EVALUATION OF THE OCCUPATIONAL HEALTH HAZARDS OF NITROGLYCERIN USING MAMMALIAN MODELS

Final Report

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A system was designed and constructed to allow monitoring of (a) blood flow in the coronary artery by the use of Doppler flow probes and (b) ventricular pressure by the use of implantable pressure transducers. The information is transmitted via implanted FM transmitters to external receivers, where the signal is processed and transformed to calibrated pressure and flow on a strip-chart recorder. This technique was used to measure the effects of inhaled or

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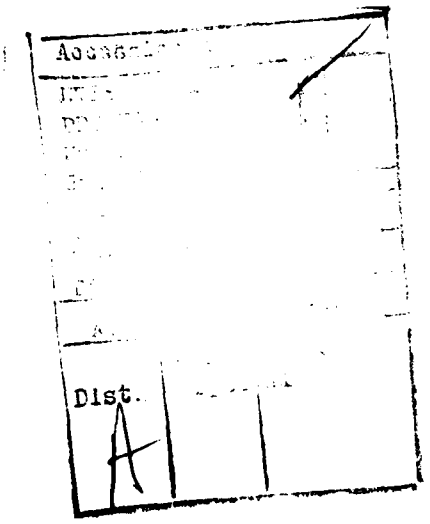
percutaneously administered nitroglycerin on coronary flow, left ventricular pressure, and ECG in dogs. The results indicated that the technique was valid for investigating whether nitroglycerin causes increased coronary flow and whether compensatory or reflex vasoconstriction occurs upon withdrawal. Our studies showed that dogs exposed to nitroglycerin by inhalation demonstrated those phenomena slightly, but that dogs treated percutaneously with 1.0 g of nitroglycerin daily did not show withdrawal symptoms. Indeed, the percutaneous treatment produced marginal changes in pressure and flow, slowing of the heart rate, and a progressive deterioration of the ECG patterns (T-wave inversion, arrhythmias, diminished atrial beats, and preventricular contractions). Studies on uptake of tritiated nitroglycerin confirmed that nitroglycerin was absorbed through the skin, but it could not be detected in the blood; however, di- and mononitroglycerins were detected in the blood. Following 10 days of daily treatment, the half-life of dinitroglycerins in blood was nearly doubled.

We could not determine a reflex vasoconstriction upon withdrawal from nitroglycerin treatment by this method. Perhaps these postulated effects cannot be demonstrated by this method or with this experimental animal model.



EXECUTIVE SUMMARY

The objective of this research was to describe the cardiovascular sequelae resulting from chronic exposure of conscious dogs to nitroglycerin. The dogs were equipped with totally implantable telemetry packs for determining coronary arterial flow via Doppler flow probes and left ventricular pressure with solid state pressure transducers. A lead II electrocardiogram was also obtained routinely from each dog. Nitroglycerin was administered subcutaneously or via inhalation using a dosing schedule designed to mimic that faced by workers in dynamite plants. Cardiovascular measurements were obtained repeatedly in these dogs both during and following nitroglycerin exposure. Emphasis was placed on detecting changes that would reflect the onset of tolerance and cardiotoxic phenomena occurring upon withdrawal from nitroglycerin treatment (i.e., the reflex vasoconstriction that is postulated to occur upon nitroglycerin withdrawal). Pharmacokinetic studies were also carried out on these instrumented dogs.

Beagles were used in the initial phase of this project. These animals were difficult to instrument because of their comparatively small size. We next studied mongrel dogs that were selected for their overall good health and suitable size. The results indicated that the technique was valid for investigating whether nitroglycerin causes increased coronary flow and whether compensatory or reflex coronary vasoconstriction occurs upon withdrawal. Our studies showed that dogs exposed to nitroglycerin by inhalation were only slightly affected by the drug, but that dogs treated percutaneously with 1.0 g of nitroglycerin daily did not show withdrawal symptoms. Indeed, the percutaneous treatment produced marginal changes in LV pressure and coronary artery flow, slowing of the heart rate, and a progressive deterioration of the ECG patterns (T-wave inversion, arrhythmias, atrial abnormalities, and premature ventricular contractions). Studies on uptake of tritiated nitroglycerin confirmed that nitroglycerin was absorbed

through the skin, but it could not be detected in the blood; however, di- and mononitroglycerins were detected in the blood. Following 10 days of daily treatment, the half-life of dinitroglycerins in blood was nearly doubled.

We could not demonstrate a reflex vasoconstriction upon withdrawal from nitroglycerin treatment by this method. Perhaps these postulated effects cannot be demonstrated by this method or with this experimental animal model.

FOREWORD

All animal facilities described in this report have been accredited by the American Association for the Accreditation of Laboratory Animal Care. Maintenance and research practices in the use of laboratory animals are conducted according to the principles and standards contained in the Guide for the Care and Use of Laboratory Animals, 1972, prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. SRI also complies with the DHEW policy on protection of animals used in research, training, or other activities as outlined in Chapter 1-43 of the DHEW Grants Administration Manual, May 14, 1973.

ACKNOWLEDGMENTS

This research effort was carried out through the efforts of the following people. Drs. Henry Allen and James Knutti, Applied Electronics Laboratory, Stanford University, provided the design, construction, and maintenance of probes, electronics, and associated hardware. Dr. Clifford Coon, Physical Sciences Division, SRI, synthesized the ^{3}H-TNG. Dr. Chozo Mitoma (SRI) did all of the absorption, distribution, and excretion studies with the ^{3}H-TNG. Dr. Ronald Spanggord and Mr. Rodney Kick (both of SRI) provided analytical support and supervision of the GC, LC, and TEA analyses of TNG in blood. Dr. Richard Jensen (SRI) provided support in cardiovascular pharmacology. Dr. John Schroeder, Division of Cardiology, Stanford University School of Medicine, served as a special consultant and reviewed our most pertinent data. Dr. Daniel P. Sasmore (SRI) provided pathology support. Finally, Ms. Sally Sorenson (SRI) provided the lead technical support for this project. Her efforts were invaluable in this entire project.

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INTRODUCTION

The toxicology and occupational health hazards of nitroglycerin,* which has been used as a coronary vasodilator for many years, have been reviewed by Dacre and Rosenblatt (1) and by Shiotsuka (2) and will not be reiterated here.

Recently there has been some concern that workers in ammunition manufacturing plants may develop a tolerance to nitroglycerin while working and then suffer episodes of coronary insufficiency and/or sudden death upon withdrawal (vacation, transfer, termination) due to a postulated reflex coronary artery vasoconstriction mechanism.

Under Contract No. DAMD 17-76-C-6068, SRI International investigated the potential tolerance and reflex vasoconstriction during withdrawal (under conditions of mild exercise and normal activity), using dogs. Specifically, we designed and developed coronary artery flow probes and pressure transducers with implantable telemetry devices. These were implanted in dogs, which were then exposed to nitroglycerin vapors or treated percutaneously with nitroglycerin daily for 10 days at a time. This report contains details of our studies and our conclusions and recommendations. Some problems that were encountered during our studies are discussed in the Appendix.

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* The expressions nitroglycerin, trinitroglycerin, and TNG are used interchangeably in this report.

MATERIALS AND METHODS

Bioengineering

The bioengineering equipment was designed and assembled especially for these studies by personnel of the Stanford University Applied Electronics Laboratory. Figure 1 shows the basic system. It consisted of implanted ultrasonic electronic flow probes, either an external (hard-wired) or an internal (implanted transmitter) telemetry link, and external Doppler processing electronics. The implantable electronics operated from a 2.8-volt, implantable, lithium battery, with either a magnetic or an Rf switch that allowed the system to be activated only data collection, thus conserving battery power. Later versions had switches that turned off automatically after 2 or 4 minutes of transmission.

For the flow probes, microcircuit techniques were used to assemble a small $(2\ mm)^2$ slab of piezoelectric ceramic into a biocompatible transducer assembly. The connections were made initially through small gold straps (later, silver was used) between the transducer faces and the stainless-steel cable used to carry signals to either the external circuitry (in the hard-wired animals) or to the implanted telemetry package. The ceramic element was sandwiched between matching and backing layers of epoxy to provide efficiency and biocompatibility. The stainless-steel cables (as well as the implantable telemetry and power supply) were coated with Silastic after initial encapsulation in wax.

A wide-band FM telemetry link was used to transmit the Doppler shift information from the implanted electronics to the external receivers. Initially, this telemetry link was external to the dog, but later it was also implanted just beneath the skin. A two-transistor discrete transmitter (200 mHz medical telemetry band), with a range of greater than 3 meters, was used. This band was chosen because of

BLOCK DIAGRAM

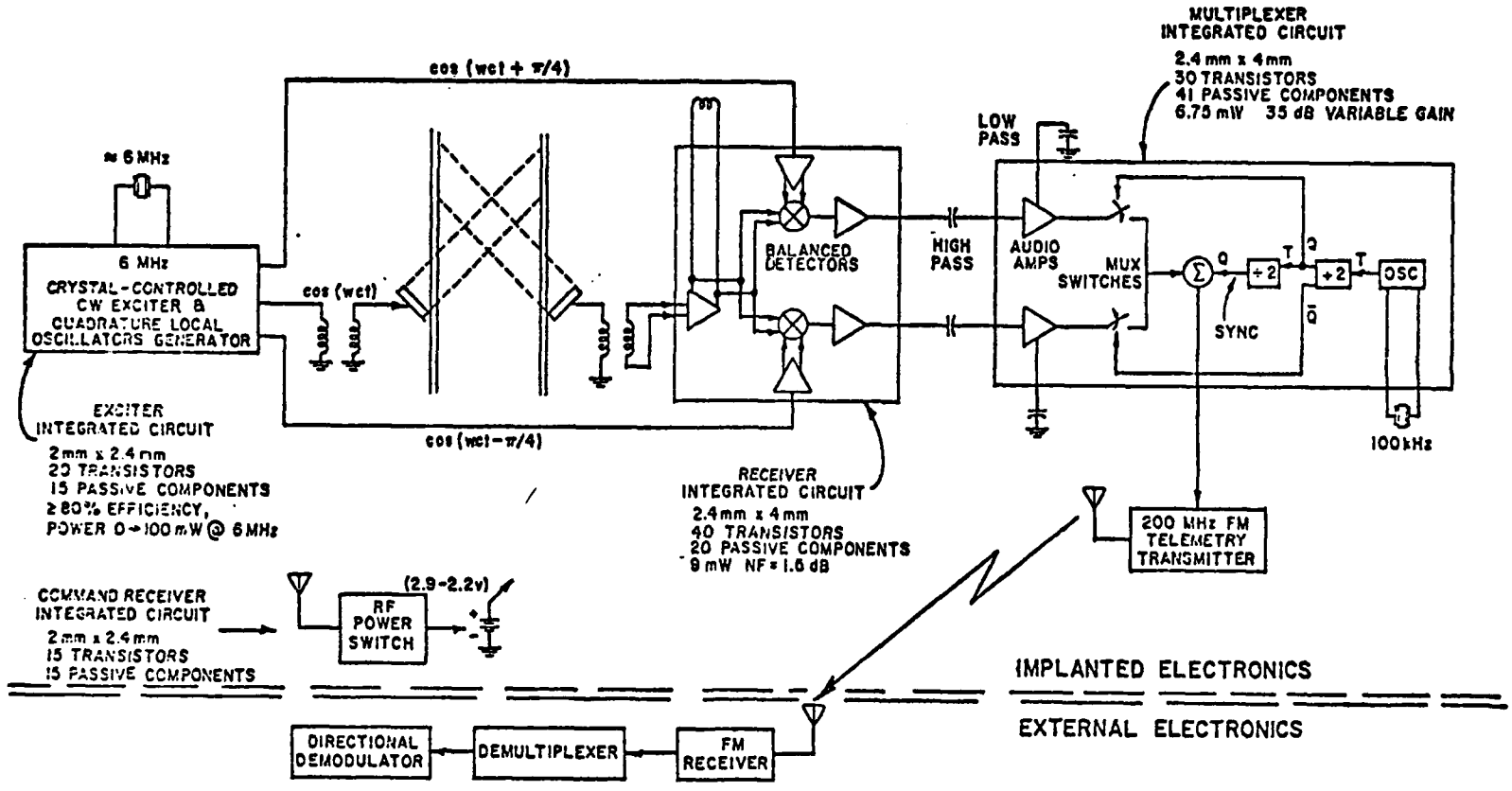


FIGURE 1 BLOCK DIAGRAM OF CW DOPPLER FLOWMETER

its normally low background noise. The signal was received on an FM telemetry receiver, which recovered the multiplexed Doppler information.

Figure 2 shows the implantable flowmeter with coronary flow transducer. The cylindrical package contains the battery and power switch assembly. The square package is the transmitter assembly with two Doppler leads and two antennae coming off the top. The battery and transmitter assembly were implanted beneath the skin but outside the thoracic cavity. The flow probes were attached to a strap and a semicircular silastic net to aid in attachment of the probe on the surface of the heart and around the coronary artery.

External signal processing electronics demultiplexed the Doppler signals from the telemetry receiver. Further signal processing filtered these signals and, through frequency-to-voltage conversion, converted them into a bidirectional flow signal for recording or display. A calibration of the system is shown in Figure 3.

A multichannel telemetry system was used to provide internal electrocardiogram (ECG) and left ventricular pressure data. The basic system is shown in Figure 4. The implanted package was identical in size to the Doppler flowmeter shown in Figure 2, with the exception of the transducers. There were two ECG transducers and one Konigsberg-type P21 piezo-resistive Wheatstone bridge pressure transducer. The external signal processing electronic hardware was the same as that used for the Doppler flowmeter.

Animals

Beagle dogs used for the initial phase of these studies were obtained from Marshall Research Farms, North Rose, New York. Later, mongrels were obtained from Knudson Kennels, Lathrop, California, and Labrador dogs were obtained from Bar-Wan, Crocker, Missouri. The beagles and Labradors, which were approximately one year old, were quarantined for a minimum of 3 weeks after arrival at SRI. Mongrels were quarantined for at least 4 weeks after arrival. During the quarantine period, the animals were observed daily and examined by a

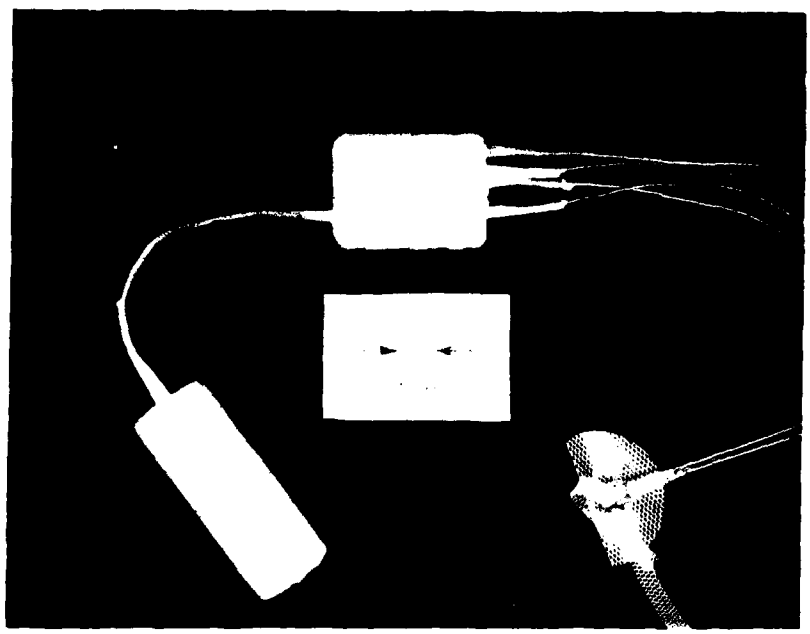


Figure 2. THE IMPLANTABLE CW DOPPLER FLOWMETER WITH CORONARY FLOW TRANSDUCER. The cylindrical package contains the battery and power switch assembly.

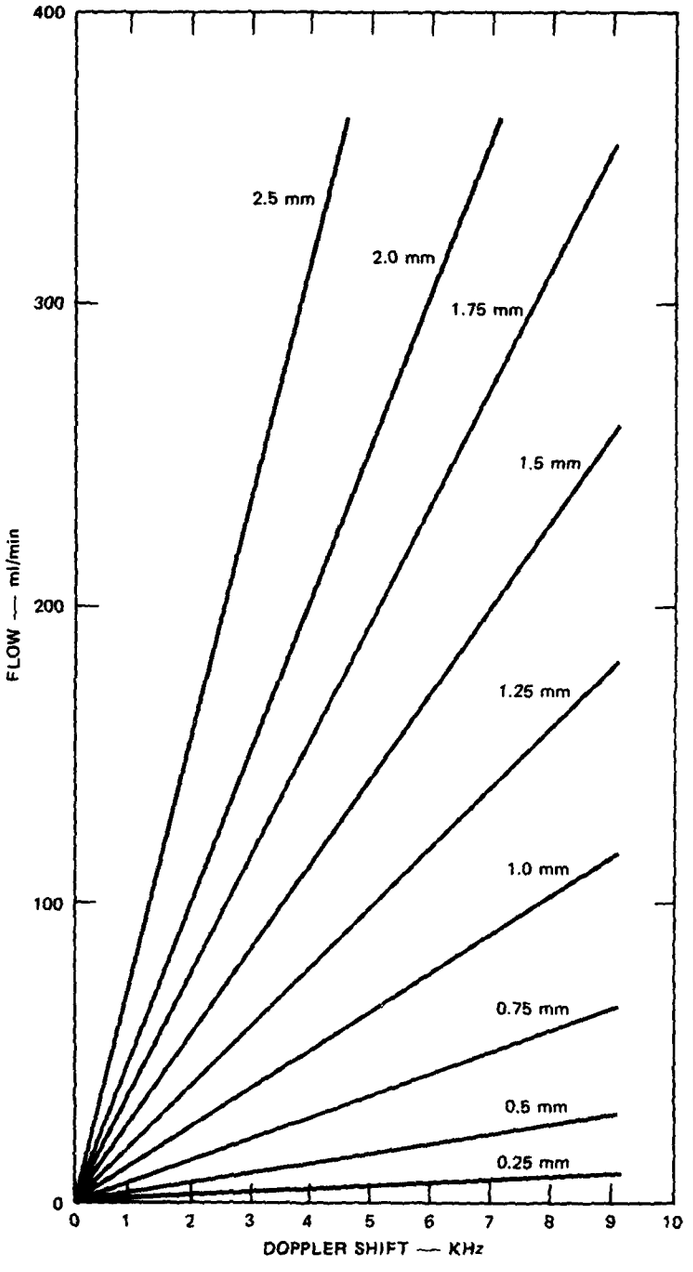
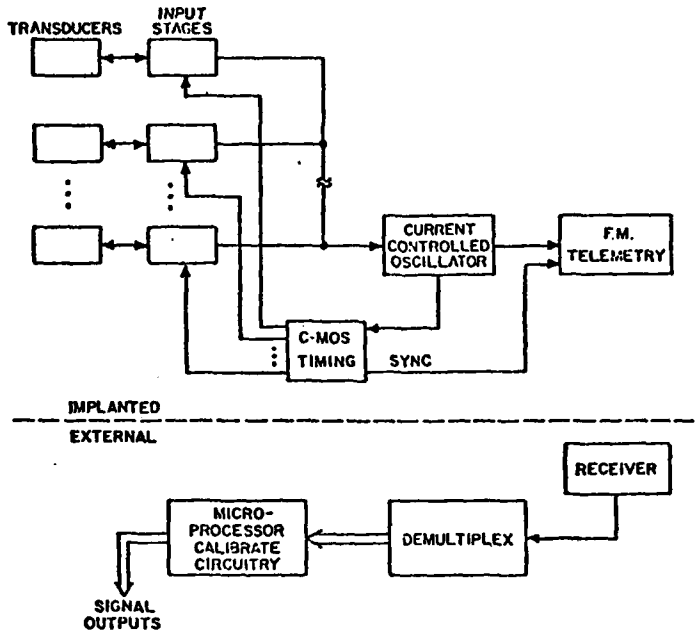


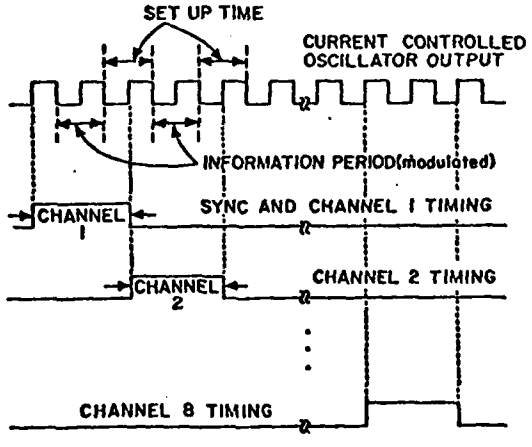
FIGURE 3 CALIBRATION FOR BLUNT PROFILE OR UNIFORM ILLUMINATION

1 KHz = CAL = 27 cc/sec $\Theta = 60^{\circ}$

Maximum Vessel Diameter = 2.5 mm.



(a) Block diagram.



(b) Associated timing.

Figure 4. MULTICHANNEL ECG PRESSURE AND TEMPERATURE TELEMETRY SYSTEM.

staff veterinarian to ensure that only healthy dogs (i.e., those with no obvious outward signs of illness and resting heart rates of 60-80 beats/minute) were used for the study. All animals were treated for parasites during this period.

Surgery

All animals were bathed and then clipped free of hair over the thoracic area the day before surgery was scheduled. They were then returned to a clean cage and fasted overnight.

On the day of surgery, a dog was anesthetized with 30 mg/kg sodium pentobarbital, given intravenously. An intravenous drip of normal saline was established and maintained throughout the surgical procedure. Subsequent administration of pentobarbital to maintain the proper level of anesthesia was through this established drip. The dog was then intubated with an endotracheal tube and placed on a Harvard respiratory pump with 100% oxygen.

After a dog was anesthetized, the skin over the thoracic area was scrubbed vigorously with Phisohex, sprayed with tincture of Zepharin (1:1000), and draped with sterile linen. An incision approximately 15 to 20 cm long was made on the left side over the sixth intercostal space. Care was taken to minimize the cutting of muscles, thus enhancing the postoperative recovery rate. The rib cage was opened at the sixth intercostal space and retractors were placed to hold the ribs apart. The pericardium was opened and suspended around the intercostal incision with #4-0 silk suture in order to present the heart in the most favorable aspect.

The apex of the heart was reflected upward and supported by two wooden tongue-depressors. Opposing purse-string sutures of #4-0 silk were placed around a circle (7-8 mm diameter). A stab wound was made through the circle and into the apex of the left ventricle with a #11 knife blade. The wound was enlarged by blunt dissection with a large, straight Kelly forcep. The pressure transducer was inserted through this wound and positioned well inside the left ventricle and then

secured in place with the purse-string sutures. The ends of these sutures were also used to secure the lead wires to the myocardium.

The left anterior descending coronary artery was freed from the myocardium over a length of 1.2-1.5 cm by blunt dissection. A Silastic strap 4-5 mm wide was placed under the artery, which was then lifted up between the two Doppler transducers. Silastic flaps on top of the flow probe, as well as the straps around the artery and the probe, were secured to the myocardial surface with #4-0 silk suture and a 3/8 circle atraumatic needle.

Surface ECG leads were sutured to the surface of the left atrium and left ventricle with #4-0 silk suture.

The pericardium was closed with #4-0 silk suture; care was taken to avoid disturbing the vagus nerve. The leads were brought out through the sixth intercostal space and secured to the rib with #1 silk suture. The intercostal space was closed with doubled #1 silk suture, and the muscle and connective tissue were resected with #3-0 chromic suture. Telemetry packages, if present, were inserted beneath the skin; the antennae were spread beneath the skin and secured in place with suture. Finally, the skin was closed with either monel steel wire or #1 silk suture.

All dogs were treated routinely with either penicillin-streptomycin or ampicillin for 5 days following surgery. Postoperative infection was not a problem in these studies, although some evidence of rejection of the implants was seen occasionally.

Generation of Nitroglycerin Vapors

Each of four glass cylinders, measuring approximately 10 cm x 30 cm, was filled with 200 g of loosely packed 10% nitroglycerin on lactose. Each end of the cylinder was stoppered with fiberglass wool packing and a #13 rubber stopper. A 0.5-inch glass tube in each rubber stopper provided an inlet and outlet for the generator tubes. Four of these tubes were connected in series so that compressed air could be passed through them and then into the inhalation chamber.

The combined outlet from the four tubes in series was directed into an inhalation chamber measuring $(76\ cm)^3$. The chamber effluent was directed out through a solution of sodium hydroxide-sodium hydrosulfide, which destroys nitroglycerin. The inhalation chamber was constructed of plywood and lined with a layer of fiberglass resin so that it could be easily cleaned during the study and destroyed by burning at the end of the project.

Percutaneous Exposures

Initially, for percutaneous administration, weighed quantities of 10% nitroglycerin on lactose were spread over a $(7.6\ cm)^2$ gauze pad. However, to increase the total absorption area, most studies were carried out using a $(10\ cm)^2$ pad. The pad was placed on the skin over the lumbar area of the dogs. This area was clipped free of hair as often as necessary. The gauze patch was covered with heavy-duty aluminum foil. The gauze and foil were held in place by wrapping them with roller gauze bandage or an elastic bandage and tape.

Coronary Flow Measurements

Figure 5 provides a typical baseline recording of coronary blood flow and an accompanying ECG pattern obtained in the first mongrel dog implanted in our laboratory. This animal was hard-wired for coronary flow but not for pressure measurements. Telemetry was not used on this animal.

Both mean and pulsatile coronary flow are shown in cm/sec (blood flow velocity). The relationship between flow velocity and volume flow in the Doppler system is linear as long as the cross-sectional area of the vessel within the transducer remains constant (3-6). We confirm this linear relationship between blood flow velocity and volume flow by means of timed collections of blood flow. At autopsy, we determine that the coronary vessels are firmly adherent to the flow transducers through fibrous scars; firm adherence minimizes changes in the cross-sectional area of the vessel within the flow transducer.

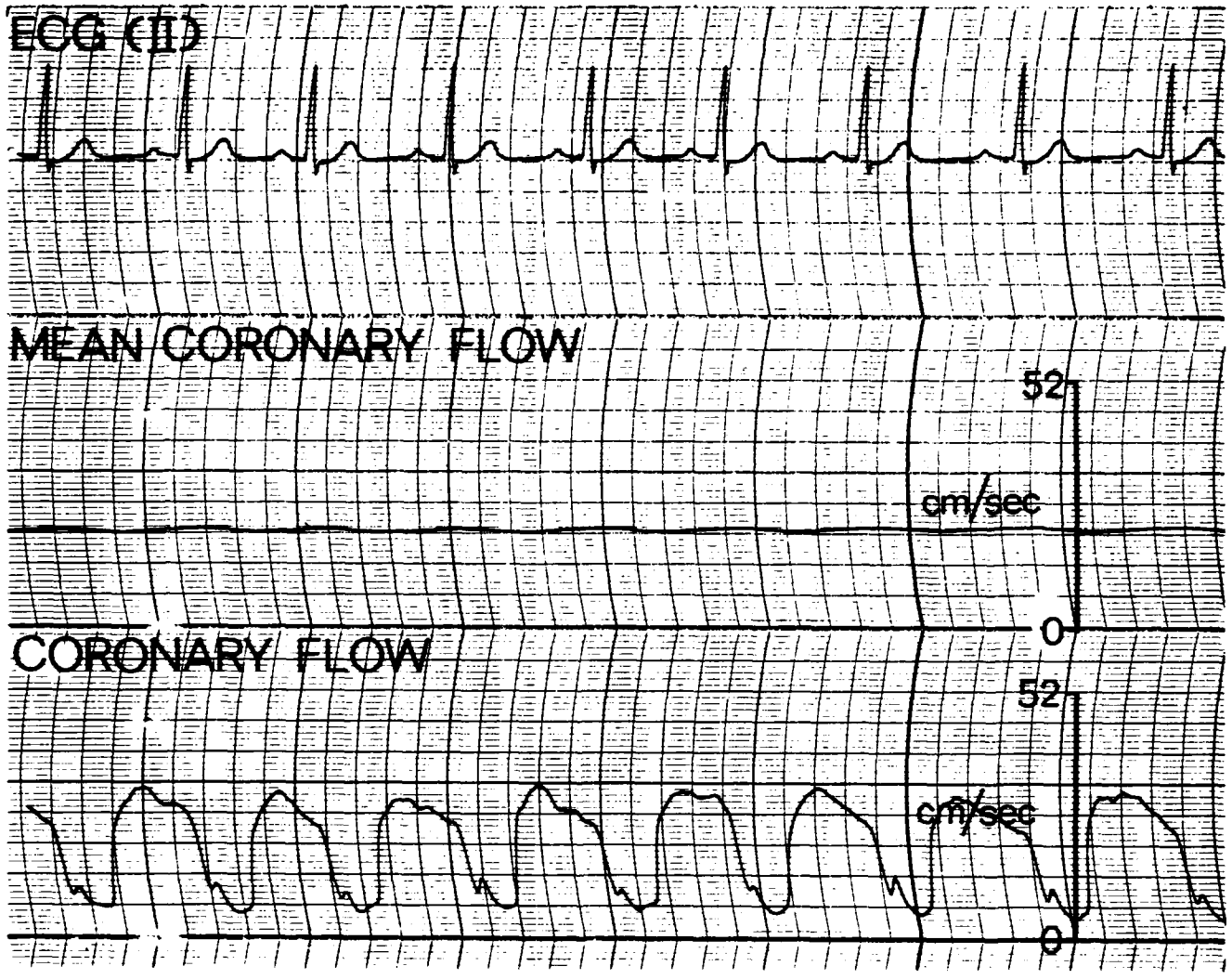


FIGURE 5 BASELINE RECORDING OF CORONARY BLOOD FLOW AND ECG PATTERN OF FIRST MONGREL DOG IMPLANTED WITH TWO PACKAGES

Coronary resistance (mm Hg/ml min^{-1}) is calculated from aortic mean pressure and mean coronary flow (volume flow). Because we did not have a pressure measurement on this animal, we could not calculate coronary resistance. By estimating aortic pressure at 105 mm Hg and coronary volume flow at 45 ml/min (calculated from estimated cross-sectional area of coronary artery made at time of implant), we arrived at a value of 2.33 mm Hg/ml min^{-1}, which is a reasonable value for baseline coronary resistance.

Nitroglycerin Analysis

Atmospheric concentrations of trinitroglycerin were determined by gas chromatography (Varian Model 3740 equipped with an electron capture detector). A 200 cm x 2 mm glass column was used that was packed with 3.5% QF-1 on Gas Chrom Q, 80-100 mesh. The column and injector temperature was 160° C; the detector temperature was 200° C. Nitrogen was the carrier gas, with a flow of 32 ml/min.

The above procedure was found to be unsuitable for extracted blood samples because interfering substances contaminated the column and the detector.

Nitroglycerin in blood was detected by liquid chromatography and a thermal energy analyzer. The liquid chromatograph was a Spectra-Physics Model 3500 with an S.60 column (EM Labs) with precolumn. The flow rate was 1.6 ml/min, using 60%/40% (v/v) isooctane/acetone for 15 min and then 70%/30% (v/v) acetone/isooctane for 5 min. The thermal energy analyzer, Model TEA 502/LC, was used under the following conditions: furnace at 500° C, 5 ml/min O_2, 15 ml/min A_2, attenuation = 4, and vacuum about 1.3 torr. The integrator was a Hewlett-Packard Model 3380A, with an attenuation of 4, slope sensitivity of 1.00, a 2-min delay, and a stop at 30 min.

Preparation of Tritiated Nitroglycerin

Tritium-labeled nitroglycerin was prepared as follows. Labeled glycerin was obtained from New England Nuclear Corporation [2-^{3}H(N)] as a solution of 115 mg in 25 ml of sterile water. The water was removed under pressure before use in this synthesis.

A solution of 0.97 g (15.4 mmol) of 100% nitric acid in 10 ml of dichloromethane was added to a 50-ml round-bottom flask that contained 2.3 g (15.3 mmol) of trifluoromethanesulfonic acid. A viscous white solid appeared immediately at the bottom of the flask. A 103.5-mg (1.12 mmol) sample of tritiated glycerin dissolved in 0.4 g of anhydrous methanol was added to the above mixture, and the reaction was stirred for 2.5 hr at room temperature. The resulting two-phase mixture was allowed to stand for 15 min. The upper phase was separated. The lower phase was washed two times with 20 drops of dichloromethane and then combined with the upper phase. The combined liquid was passed through a silica gel and "Woelm" aluminum oxide (basic activity grade 1) column. The evaporation of one-tenth weight of the filtrate under vacuum yielded 10.2 mg of viscous, light, green-yellow liquid. The infrared spectrum was identical with that of an authentic sample of nitroglycerin. The total yield based on this aliquot was 40%. The specific activity of the synthesized TNG-2^{3}H was 200 mCi/mmol.

Blood Levels of Nitroglycerin

For determinations of unlabeled nitroglycerin in blood by the thermal energy analyzer, a sample consisting of 200 ml of blood, 100 µl of 1 M Ag NO_3, and 1056 or 528 ng of dipropylnitrosamine (as an internal standard) was extracted with one 2-ml aliquot and five 1-ml aliquots of ethyl acetate. The extraction was accomplished by rapid injection of the ethyl acetate into the sample. After mixing, the organic layer was removed by syringe. The extracts were combined and passed through a column of anhydrous sodium sulfate. The dried extract was concentrated to 500-1000 µl, using a gentle stream of nitrogen.

Blood levels of tritiated nitroglycerins were determined by separation of the mono-, di-, and trinitroglycerins on silica gel plates and elution and quantitation using a liquid scintillation spectrometer.

Precoated silica gel 60 plates (with fluorescent indicator, 0.25 mm thickness, E. M. Laboratories, Inc., Elmsford, New York) were used for all experiments. Samples were spotted at 2.0 cm and developed for a minimum of 10 cm. The solvent used was benzene:ethyl acetate (4:1). The Rf of TNG, 1,3-DNG, 1,2-DNG, and MNG were 0.75, 0.54, 0.36, and 0.06, respectively.

Blood samples (2 ml) were drawn into a solution of 0.5 ml of 5% $HgCl_2$ to stop all enzymatic activity of serum and red cells from further degradation of nitroglycerins. The samples were extracted twice into 5 ml each of ether. The ether was blown off and the residue was (1) dissolved in 0.1-ml EtOAC, 10 1 spotted on silica gel plates for TLC in benzene:EtOAC (4:1), or (2) saved for GC analysis. Ether extracts the trinitroglycerin and dinitroglycerins quantitatively but the mononitroglycerins only to the extent of 65%. Therefore, a correction factor was used to calculate the concentrations of mononitroglycerins.

Pathology

Tissue sections of the coronary artery and the adjacent myocardium were taken from dogs when the probes were removed after they were no longer functional. The cross sectional area (A) of the artery was determined for converting velocity flow to volume flow by the following: volume flow (ml/min) = velocity flow (cm/sec) x A^2. The tissues were fixed routinely in 10% neutral buffered formalin and stained with eosin and hematoxylin.

RESULTS

Thirty-four dogs were used in this study. Table 1 lists the type of implanted hardware each dog received and the success or failure of each implant.

Preliminary Studies

To determine the minimum dose of nitroglycerin necessary to elicit a detectable response in coronary flow, an instrumented beagle weighing 10 kg was injected intravenously with 1.0 mg of nitroglycerin in 50% ethanol (10 mg of nitroglycerin per milliliter of solution). An immediate increase in mean coronary flow was observed, with an increase in the magnitude of the pulsatile flow. A similar volume of 50% ethanol produced just the opposite effect. After its coronary flow returned to baseline, the dog was injected with 2.5 mg of nitroglycerin (TNG). Figure 6 shows the effects of this injection. The magnitude of the pulsatile coronary flow promptly increased, and the mean flow rate nearly doubled. Therefore, this system apparently can detect changes in coronary flow produced by intravenous nitroglycerin. The minimum dose necessary to elicit a response (e.g., 5-10% increase in flow) in this 10-kg dog was found to be 0.125 mg.

Two dogs were exposed to nitroglycerin vapors in the inhalation chamber for 1 hour. During the exposures, the magnitude of the pulsatile flow increased as the exposure progressed, indicating that some effect was obtained from the inhaled nitroglycerin vapors. However, we had some difficulty in obtaining adequate chamber samples for concentration analysis, so the atmospheric concentration of nitroglycerin generated in later experiments was increased to the maximum considered safe.

Dog NG-8 was exposed daily for 6 hours to nitroglycerin vapors for 10 days. The vapor concentrations in the chamber, shown in Table 2,

Table 1

DOGS IMPLANTED WITH CORONARY FLOW PROBES AND
LEFT VENTRICULAR PRESSURE PROBES WITH TELEMETRY PACKAGES

Dog No.	Implant	Remarks
NG-1	Hard wire	Probe not functioning after 60 days
NG-2*	Hard wire	Dog chewed off leads
NG-3	Hard wire	Dog chewed off leads
NG-4*	Hard wire	Dog chewed off leads
NG-5	Telemetry package	Died of unknown causes
NG-6*	Telemetry package	Acute studies
NG-7	Telemetry package	Acute studies
NG-8*†	Telemetry package	Used for 10-day inhalation study
NG-9	Hard wire	Acute studies; dog chewed off leads
NG-13	Telemetry package	Transmitter failure (dead battery?)
NG-15†	Hard wire	10-Day dermal study
NG-17†	Telemetry package	10-Day inhalation study
	Battery replaced	10-Day dermal study
NG-19	Hard wire	Probe failure
NG-21†	Telemetry package	10-Day dermal study
NG-23	Telemetry package	Dog did not recover from surgery
NG-24	Telemetry package	Dog did not recover from surgery
NG-25	Telemetry package	Electronics failure
NG-26	Hard wire	Probe failure
NG-28	Telemetry package	Electronics failure
NG-29	Telemetry package	Dog died 24 hr postoperatively
NG-30	Telemetry package	Electronics failure, poor health postoperatively
NG-31	Telemetry package	Dog died postoperatively
NG-32	Telemetry package	Dog was in poor health postoperatively; necropsy revealed old nephritis scars with loss of 70% of kidneys
NG-33†	Telemetry package	28-Day study
NG-34†	Telemetry package	28-Day study
NG-35†	Telemetry package	28-Day study
NG-36	Telemetry package	Dog died during surgery
NG-37	Telemetry package	Electronics failure (batteries)
NG-38	Telemetry package	Dog never recovered from surgery
NG-39†	Telemetry package	28-Day study
NG-40	Telemetry package	Electronics failure
NG-41†	Telemetry package	28-Day study
NG-42	Telemetry package	Electronics failure
NG-43†	Telemetry package	28-Day study plus 10-day control data

* Females. All other dogs used in this study were males.
† Successful studies.

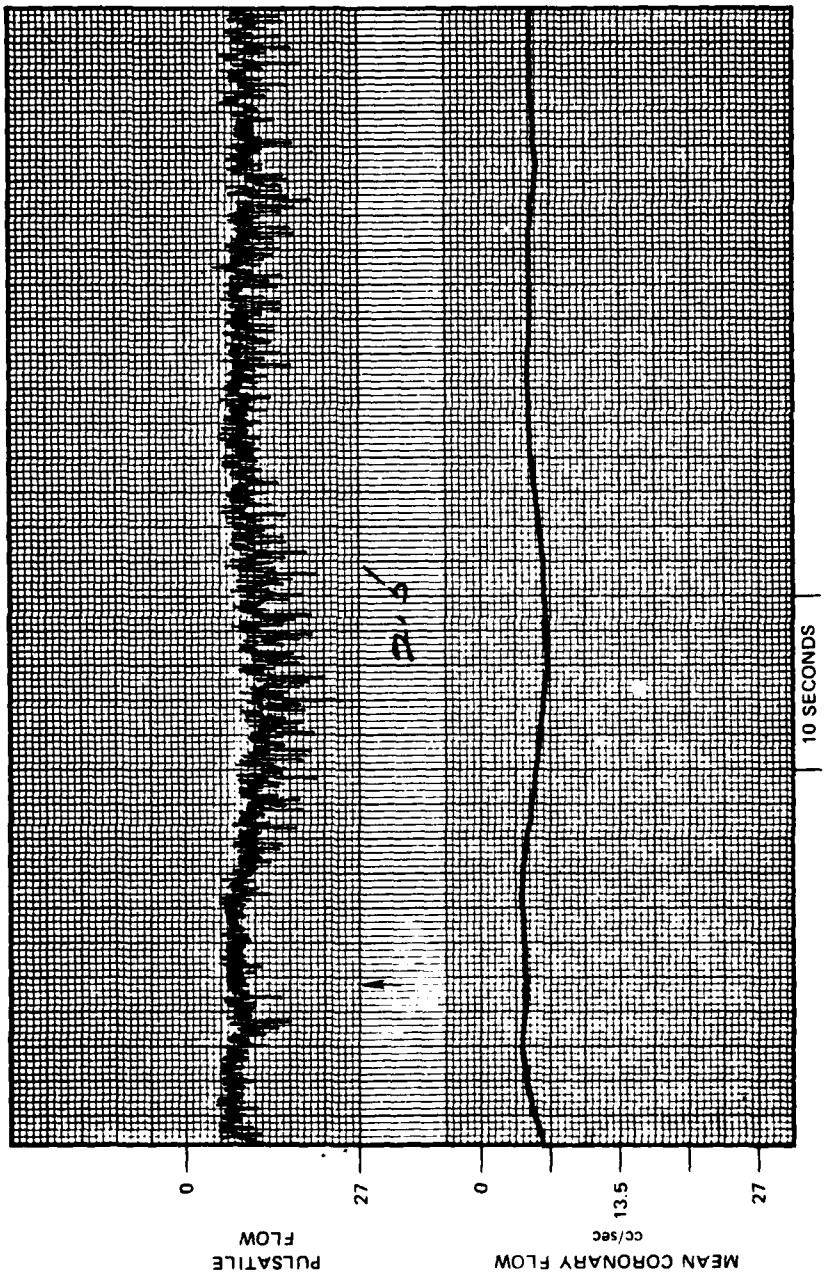


FIGURE 6 PULSATILE FLOW AND MEAN CORONARY FLOW IN A DOG AFTER THE INTRAVENOUS INJECTION OF 2.5 mg OF TRINITROGLYCERIN

Table 2

DAILY CHAMBER CONCENTRATION OF NITROGLYCERIN
DURING EXPOSURE OF DOG NG-8
(mg/m^3)

Day	Mean Concentration and Range
1	0.202 (0.10-0.21)
2	0.151 (0.01-0.43)
3	0.29 (0.02-1.44)
4	0.227 (0.01-1.4)
5	0.283 (0.01-2.32)
6	0.31 (0.02-1.91)
7	0.02
8	0.314 (0.06-0.54)
9	0.324 (0.05-1.70)
10	0.221 (0.04-0.25)

were extremely low and variable while the desired concentrations were going into the chamber (~5 mg/m^3). Apparently, either the dog absorbed a great amount of the nitroglycerin on its fur or some nitroglycerin adhered to and deposited on the fiberglass, making prediction of inhaled doses nearly impossible. We infer this because the size of the chamber dimensions was kept small in order to minimize the possibilities of an explosion from nitroglycerin accumulating in it during the exposure and the relative surface area of the dog was greater than ideal (7).

Table 3 summarizes the heart rate and mean coronary flow data collected on this dog before, during, and immediately after exposure each day. Because heart rate was not controlled, some coronary flow effects may be secondary to altered heart rate. This animal was doing well until the sixth day, when we were unable to activate the magnetic switch to turn on the transmitter and collect data during the exposure. On the seventh day we were unable to activate the switch at all, and on the eighth and ninth days we were able to keep it on only once. This was most unfortunate because some reduced flow was indicated on the eighth and ninth exposure days even though heart rate was greatly increased, and this in turn should elevate coronary flow. We were unable to collect any data during the recovery period. Figure 7 shows the transient increase in pulsatile and mean flow observed daily as the nitroglycerin exposures began. This was observed in both dogs given an inhalation exposure.

A second dog, NG-17, was started on the daily exposure regimen (6 hr/day for 10 days). Table 4 shows the chamber concentrations. Again, the inlet concentrations were in the desired range but the chamber concentrations were very low. After four days in the chamber concentrations increased daily, as if the dog's fur or chamber absorption sites were becoming saturated. Table 5 presents the daily heart rate and coronary flow data. Again, we had some difficulty in manipulating the magnetic switch. In general, the coronary flow seemed to be most improved (increased) from the sixth day of exposure. However, this

Table 3

HEART RATE AND CORONARY FLOW IN DOG NG-8
DURING EXPOSURE TO VAPORS OF TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Mean Coronary Artery Flow (cc/sec)	Remarks
1	102	7.1	Baseline
	90	5.1	Resting in chamber (TNG on)
	135	7.7	TNG off
2	88	7.1	In chamber
	108	8.4	TNG on
	68	6.4	Resting
	68	6.4	Resting
	75	8.4	Walking
	180	5.8 - 8.4	TNG off
	138	7.7 - 8.4	Out of chamber
3	144	6.4	Walking on floor
	88	5.8	In chamber
	104	6.4 - 7.1	TNG on
	60	5.1 - 6.4	Resting in chamber
	126	6.4 - 7.1	Chamber open (TNG off)
4	124	7.1 - 7.7	Walking
	84	6.4 - 7.1	Resting in chamber (TNG on)
	112	6.4 - 7.1	Chamber open
5	92	6.4	TNG on
	88	6.4	Resting
	100	7.1 - 7.7	TNG off
6	100	8.4	TNG on
	87	7.1 - 7.7	TNG off
7	Unusable		
8	120	4.6 - 5.3	Walking
9	--	4.0 - 4.6	In chamber (TNG on)
10	Unusable		

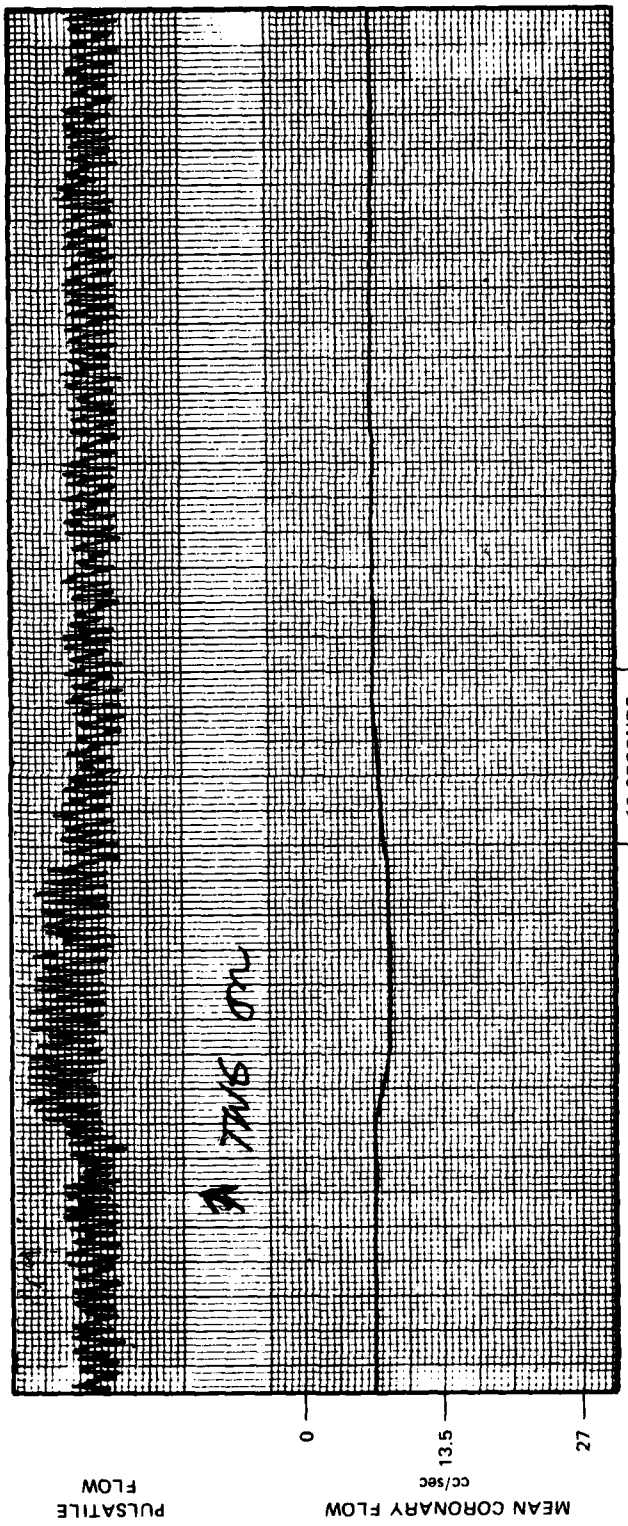


FIGURE 7 TYPICAL CHANGES IN PULSATILE FLOW AND MEAN CORONARY FLOW WHEN THE NITROGLYCERIN VAPOR GENERATORS WERE TURNED ON EACH MORNING (Dog NG-8)

Table 4

DAILY CHAMBER CONCENTRATIONS OF NITROGLYCERIN
DURING EXPOSURE OF DOG NG-17
(mg/m^3)

Day	Mean Concentration and Range	
	Inlet	Chamber
1	--	0.09 (0.01-0.27)
2	--	0.068 (0.01-0.30)
3	--	0.037 (0.01-0.08)
4	--	0.01 (0.002-0.03)
5	6.35	0.46 (0.12-0.85)
6	--	0.433 (0.19-0.96)
7	7.41	0.545 (0.30-0.76)
8	7.50	0.8
9	11.54	1.33 (1.06-1.70)
10	2.30	1.67 (0.06-3.91)

Table 5

HEART RATE AND CORONARY FLOW IN DOG NG-17
DURING EXPOSURES TO VAPORS OF TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Coronary Artery Flow (cc/sec)	Remarks
1	140	3.9 - 4.5	TNG on
	140	3.2 - 3.9	Out of chamber
2	144	4.0	In chamber
	124	2.3	TNG on
	116	3.0	Out of chamber
3	140	3.4	In chamber
	104	2.7	TNG on
	88	1.7 - 2.0	
	92	2.4	TNG off
	128	3.0 - 3.4	Out of chamber
4	160	4.6	In chamber
	96	3.3	TNG on
	88	3.3	
	184	2.6	Out of chamber
5	156	3.9	In chamber
	168	--	Out of chamber
6	156	5.3 - 6.6	In chamber
	96	4.6	TNG on
	136	5.9 - 7.3	Out of chamber
7	132	5.9	In chamber
	160	6.6	TNG on
	128	4.6	
	108	5.9	
	96	5.9 - 7.2	
	120	5.9	
	104	4.6	
	180	7.9 - 8.6	Out of chamber
	164	7.2	10 min $\overline{p}$, out of chamber
	152	5.9	20 min $\overline{p}$, out of chamber
	136	5.9	30 min $\overline{p}$, out of chamber

(Continued)

Table 5 (Concluded)

Day	Heart Rate	Coronary Artery Flow (cc/sec)	Remarks
8	144	4.7	In chamber
	164	6.8	TNG on
	136	4.1 - 5.4	
	128	4.1 - 4.7	
	116	4.1	Out of chamber
9	148	5.4	In chamber
	152	4.7 - 5.4	TNG on
	132	4.7 - 5.4	Out of chamber
10	128	4.7	In chamber
	152	4.1	TNG on
	140	3.3 - 4.1	Out of chamber
11	120	2.7	Recovery

may reflect an increase in chamber concentration during the last 6 days. On the eleventh day (24 hr after the last exposure), the coronary flow was reduced below those levels obtained at that heart rate (120) during the exposures. In fact, the coronary flow appeared to be beginning to slow during the last 2 exposure days.

Percutaneous Studies

One dog implanted with a hard-wire transducer, dog NG-15, was subjected to four separate percutaneous treatment protocols, as follows:

- Exposure 1--Total dose, 2 g of 10% nitroglycerin on lactose under an occluded percutaneous bandage; exposure of 6 hr/day for 10 days followed by 4 days of post-exposure analysis.
- Exposure 2--Total dose, 5 g of 10% nitroglycerin on lactose under an occluded percutaneous bandage; exposure of 6 hr/day for 10 days followed by 3 days of post-exposure analysis.
- Exposure 3--Total dose, 10 g of 10% nitroglycerin on lactose under an occluded percutaneous bandage; exposure of 6 hr/day for 12 days followed by 4 days of post-exposure analysis.
- Exposure 4--Total dose, 10 g of 10% nitroglycerin on lactose under an occluded percutaneous bandage; exposure of 6 hr/day for 9 days followed by 6 days of post-exposure analysis.

Daily changes in coronary blood flow and heart rate for Exposure 1 are given in Table 6.

Dog NG-15 was placed on a percutaneous treatment regimen (6 hr/day for 10 days). It was treated with 2.0 g of 10% nitroglycerin on lactose or 200 mg of nitroglycerin under an occluded percutaneous bandage (see Table 6). The changes in coronary flow were not consistent and may be obscured by alterations in heart rate. Two days after the treatment period ended, the flow was at its lowest for that heart rate. On 6 of the 10 treatment days the heart rate was depressed by the application of the TNG.

For Exposures 2, 3, and 4, we collected coronary flow data and ECG records on a daily basis at the following intervals: Pre-exposure (usually 0800 hr); at the time exposure was to begin (0845 to 0900 hr); 2 hr after application of the patch (1100 hr); 4 hr after application

Table 6

HEART RATE AND CORONARY FLOW IN DOG NG-15
DURING DERMAL EXPOSURES TO TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Coronary Artery Flow (cc/sec)	Remarks
1	156	4.0	Standing
	128	4.7 - 5.4	TNG treatment
	140	4.7	Post-treatment
2	132	3.3	Baseline
	136	4.0	Post-treatment
3	144	4.0	Baseline
	140	4.0	TNG applied
	204	4.0	
	144	3.3 - 4.0	Post-treatment
4	152	4.0	Pretreatment
	144	4.7	TNG applied
5	136	3.3	Baseline
	132	3.3	TNG applied
	136	3.3	TNG applied
6	136	2.0	Baseline
	192	4.0	TNG applied
	156	2.0 - 2.6	TNG applied
7	160	2.0	Baseline
	128	2.0	TNG applied
	156	2.0 - 2.6	TNG applied
	144	1.4	Post-treatment
8	172	3.3 - 4.0	Baseline
	148	3.3	TNG applied
	152	3.3	
	160	3.3 - 4.0	Post-treatment

(Continued)

Table 6 (Concluded)

Day	Heart Rate	Coronary Artery Flow (cc/sec)	Remarks
9	160	4.0	Baseline
	148	2.4 - 2.6	Begin treatment
	152	2.0 - 2.6	
	160	2.0 - 2.6	Post-treatment
10	168	3.4	Last day of percutaneous
	168	2.6 - 3.4	treatment
	144	2.0 - 2.6	
	164	3.4	
	160	2.7	
11			Recovery
12	136	2.0	Recovery

of the patch (1300 hr); and 1/2 hr after the end of exposure (1600 hr). This protocol was followed on days that the animal was exposed to nitroglycerin as well as on post-exposure days.

The flow data are generally not reliable for Exposures 2, 3, and 4. Both the mean and phasic flow signals became attenuated and it was difficult to separate the flow signal from background noise, but ECG analysis was done daily.

In the ECGs, there was no clear-cut evidence of myocardial ischemia or infarction in our data. There is ample evidence that percutaneous exposure to nitroglycerin leads to a sinus arrhythmia, usually a bradycardia with an irregular sinus discharge. The voltage amplitude of the QRS complex progressively decreased during the study period. It is possible that this phenomenon was caused by the compound or was simply a pathological change in the myocardium secondary to the implanted probe.

A finding that may be significant to post-exposure changes in the myocardium was observed. That is, there was a definitie change in the time and voltage characteristics of Leads I and III between Days 1 and 3 of the post-exposure period.

In evaluating the results from our first dogs (beagles), it was decided to shift to larger animals so that they would provide larger coronary arteries, and hence better flow data. We then tried mongrel dogs; however, because of their generally poor health and "normal" pathology, we soon decided to use laboratory-raised Labrador dogs.

Figure 8 illustrates the acute effects of intravenously administered TNG and verapamil (a vasodilator) on mean and pulsatile coronary flow velocity recorded from the animal described in the methods section. TNG was administered in decreasing doses in an attempt to find a threshold dose for coronary flow effects. It may be seen in the figure that TNG causes a dose-dependent increase in coronary flow velocity. Verapamil (100 μg/kg), given for comparative purposes (panel D), caused only a slight increase in coronary flow velocity; however, the duration of the increase in flow velocity substantially exceeded that caused by any dose of TNG studied.

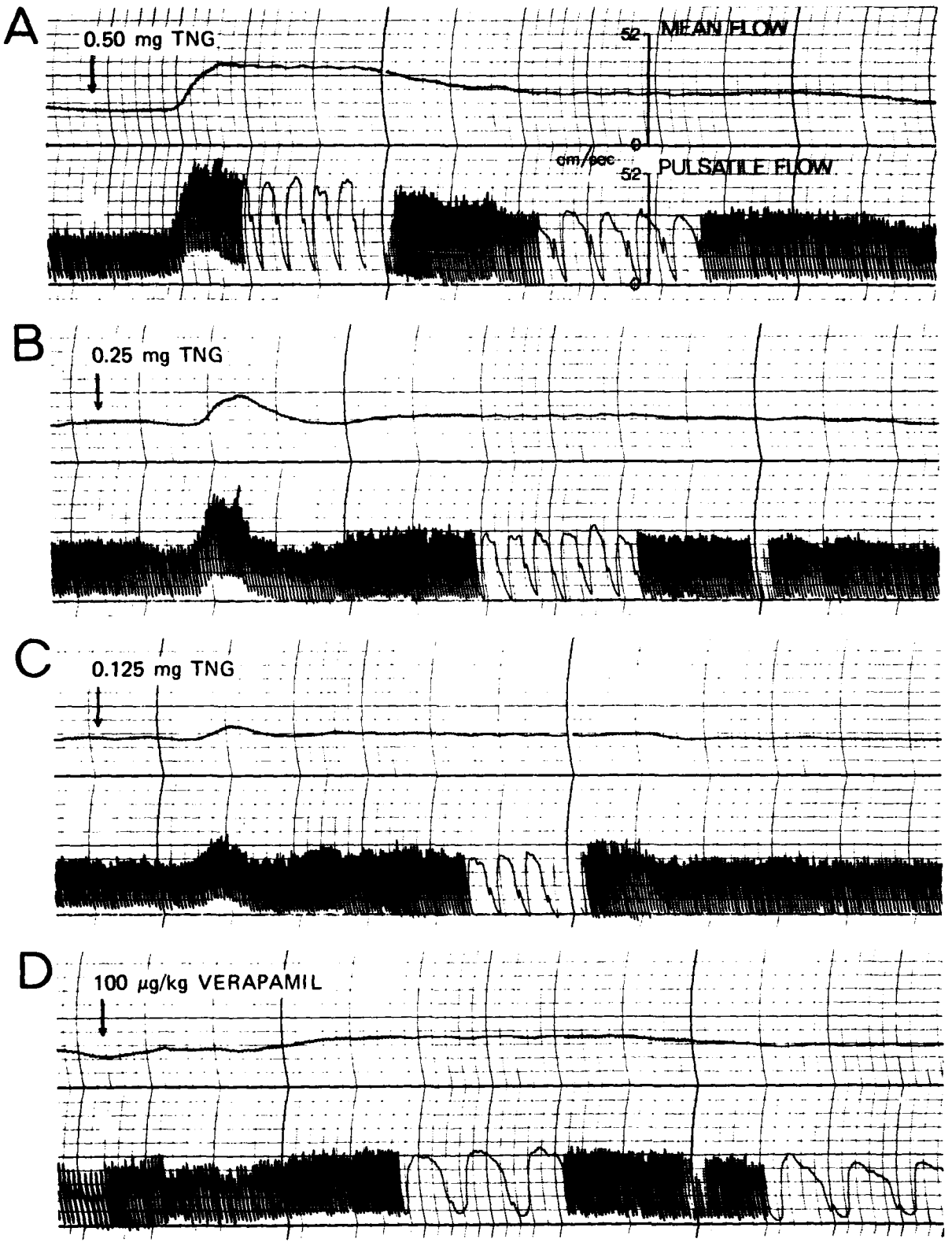


FIGURE 8 ACUTE EFFECTS OF INTRAVENOUSLY ADMINISTERED TNG AND VERAPAMIL ON MEAN AND PULSATILE CORONARY FLOW VELOCITY

Analysis of Telemetry Data

To obtain telemetry data, we exposed all the remaining dogs as follows. Baseline data were accumulated each morning, and then the TNG patch was applied to each dog. Telemetry data were collected frequently during the first half hour of treatment and then hourly on the hour for a total of 6 hr. Then the treatment was discontinued, and the final data for the day were collected 0.5 hr later. The daily treatment continued for 10 days, followed by 4 days of recovery (first series).

During recovery, telemetry data were collected frequently during each day to correspond with the data collection time periods during treatment. The entire schedule was then repeated, i.e., 10 daily treatments and 4 days of recovery (second series).

Table 7 shows the heart rate, left ventricular pressure (LVP), and mean coronary flow of dog NG-33 during daily treatment with 10 g of TNG and recovery. The table presents each daily observation--pretreatment baseline, 0.5 hr after the application of TNG percutaneously under an occluded dressing, 4 to 6 hr after the start of treatment, and 0.5 hr after cessation of the treatment.

An interesting observation in dog NG-33 was that, compared with values for days 1-7, its LVP seemed to increase during the eighth, ninth, and tenth treatment days and did not immediately recover during the recovery period. This was true for both the first and second series of treatments. Also, the LVP in this dog usually dropped each day after the treatment was started. Similarly, the mean coronary flow (MCF) usually dropped after each daily treatment began and usually rose after treatment was stopped. We do not know whether this change in coronary flow is an effect of the LVP or whether it is due to changes in the coronary artery diameter.

Table 8 shows the effect of treadmill activity on the LVP and heart rate of dog NG-33. The dog was subjected to 2.0 min of trotting at 4.75 MPH during the daily treatment, and then data were collected at 1-min intervals thereafter. Although exercise did have an effect on the ECG (improves rhythm and T-wave), there was no unexpected effect on the heart rate or LVP.

Table 7

LEFT VENTRICULAR PRESSURE, HEART RATE, AND MEAN CORONARY FLOW IN DOG NG-33 DURING AND AFTER PERCUTANEOUS EXPOSURE TO 10 g OF NITROGLYCERIN ON LACTOSE

	First Series of Exposures			Second Series of Exposures		
Exposure Day	Heart Rate	Left Ventricular Pressure	Mean Coronary Artery Flow	Heart Rate	Left Ventricular Pressure	Mean Coronary Artery Flow
1	84	96	70	72	88	EF*
	60	92	31	72	108	
	50	84	21	96	100	
	40	110	33	96	80	
2	72	84	34	84	108	
	60	--	36	72	80	
	60	80	28	72	88	
	96	84	34	66	88	
3	96	108	39	108	88	
	72	96	28	70	86	
	72	112	29	78	60	
	70	112	29	90	76	
4	96	112	37	72	60	
	80	104	31	80	62	
	60	112	26	84	70	
		104	28	78	60	
5	84	116	26	80	96	
	60	96	22	84	96	
	60	104	29	84	118	
	72	104	--	--	--	
6	80	100	39	--	--	
	60	92	28	--	--	
	60	104	26	78	108	
	72	100	41	78	110	
7	90	108	39	90	122	
	84	104	31	78	140	
	60	100	42	70	--	
	96	108	42	70	--	

(Continued)

Table 7 (Concluded)

Exposure Day	First Series of Exposures			Second Series of Exposures		
	Heart Rate	Left Ventricular Pressure	Mean Coronary Artery Flow	Heart Rate	Left Ventricular Pressure	Mean Coronary Artery Flow
8	80	--	--	80	148	EF*
	60	--	--	60	148	
	60	144	26	96	152	
		160	39	70	140	
9	108	168	23	--	--	
	84	148	18	--	--	
	102	150	--	78	136	
	114	164	--	78	136	
10	114	152	42	80	152	
	96	132	36	60	136	
	72	144	36	60	132	
		136	--	80	132	
1 Recovery	96	128	--	78	136	
	108	132	--	102	120	
	84	120		60	136	
				54	124	
2 Recovery	120	168	29	70	116	
	96	128	32	90	108	
	90	132	33	110	112	
	102	148	36	60	124	
3 Recovery	78	132	--	80	120	
	70	128	34	60	116	
	60	124	34	84	120	
	70	140	36	80	116	
4 Recovery	72	128	49	126	156	
	70	132	52	90	136	
	72	124	--	78	128	
				72	116	

* Electronics failure.
Left ventricular pressure, in mm Hg
Heart rate, in beats/minute
Mean coronary flow, in cm/sec.

Each daily reading, in order, is baseline, one-half hour after treatment began, 4 to 6 hours after treatment began, and one-half hour after the TNG was removed.

Table 8

LEFT VENTRICULAR PRESSURE AND HEART RATE IN DOG NG-33
AFTER A 2-MINUTE TREADMILL STRESS DURING TREATMENT WITH TNG

Time After Stress (minutes)	Heart Rate	Left Ventricular Pressure
Baseline	108	96
		88
Fourth treatment day, first treatment series		
1	90	144
2	108	148
Fifth treatment day, second series		
1	96	124
2	84	96
3	78	88
10	66	96
Eighth treatment day, second series		
1	103	160
2		152
1	90	156
2	84	156
4	90	152
Ninth treatment day, second series		
0	--	160
1	78	136
2	66	140
3	66	136
Tenth treatment day, second series		
1	90	140
2	96	136
3	72	124
4	72	136
First recovery day		
2	96	144
3	102	124
5	72	124
6	84	128
Second recovery day		
1	114	128
3	108	120
5	108	120
8	78	144

The LVP, heart rate, and mean coronary flow in dog NG-34 during daily treatment with and recovery from 10 g of 10% TNG on lactose, administered percutaneously, are shown in Figure 9 and Table 9. During the first exposure series, the heart rate increased within 15 minutes after treatment started on Days 1 and 8 and decreased on Days 3, 4, 6, 7, and 10. Therefore, no consistent pattern was seen relative to the treatment. On Days 1, 2, 3, 5, 6, and 8, the LVP increased immediately after the application of TNG. Unfortunately, the battery failed on the eighth day, so no further data were available. Coronary flow was generally at its lowest during the sixth treatment day of the first exposure series and at its highest during the first recovery day.

Assessment of coronary flow during the second exposure series is difficult because of the more erratic pulsatile flow patterns. There was a general decrease in heart rate on treatment days 5 through 8 and on the fourth recovery day.

Figure 9 shows the ECG, LVP, and mean and pulsatile coronary flow patterns in dog NG-34. There was a dramatic increase in LVP, LV dp/dt, and coronary flow during the beginning of the first day of treatment, as shown in the recordings at 15 minutes. The progressively erratic pulsatile coronary flow seemed to improve on the second through the fourth recovery day. The increasing irregularity in heart rate and the abnormal ECG patterns may have contributed to the changing coronary flow patterns.

Table 10 shows the heart rate and LVP of NG-35 during and after its two series of daily treatments with 10 g of 10% TNG on lactose. Very little change was evident from the treatment except for an increase in LVP during the second and third days of treatment in the first exposure series and before the fourth exposure started. Thereafter, the pressure remained relatively constant, even in the presence of large fluctuations in heart rate. This dog had a very slow heart rate and the application of TNG daily often caused a further slowing during both the first and second treatment series. The coronary flow probes were not functioning in this dog.

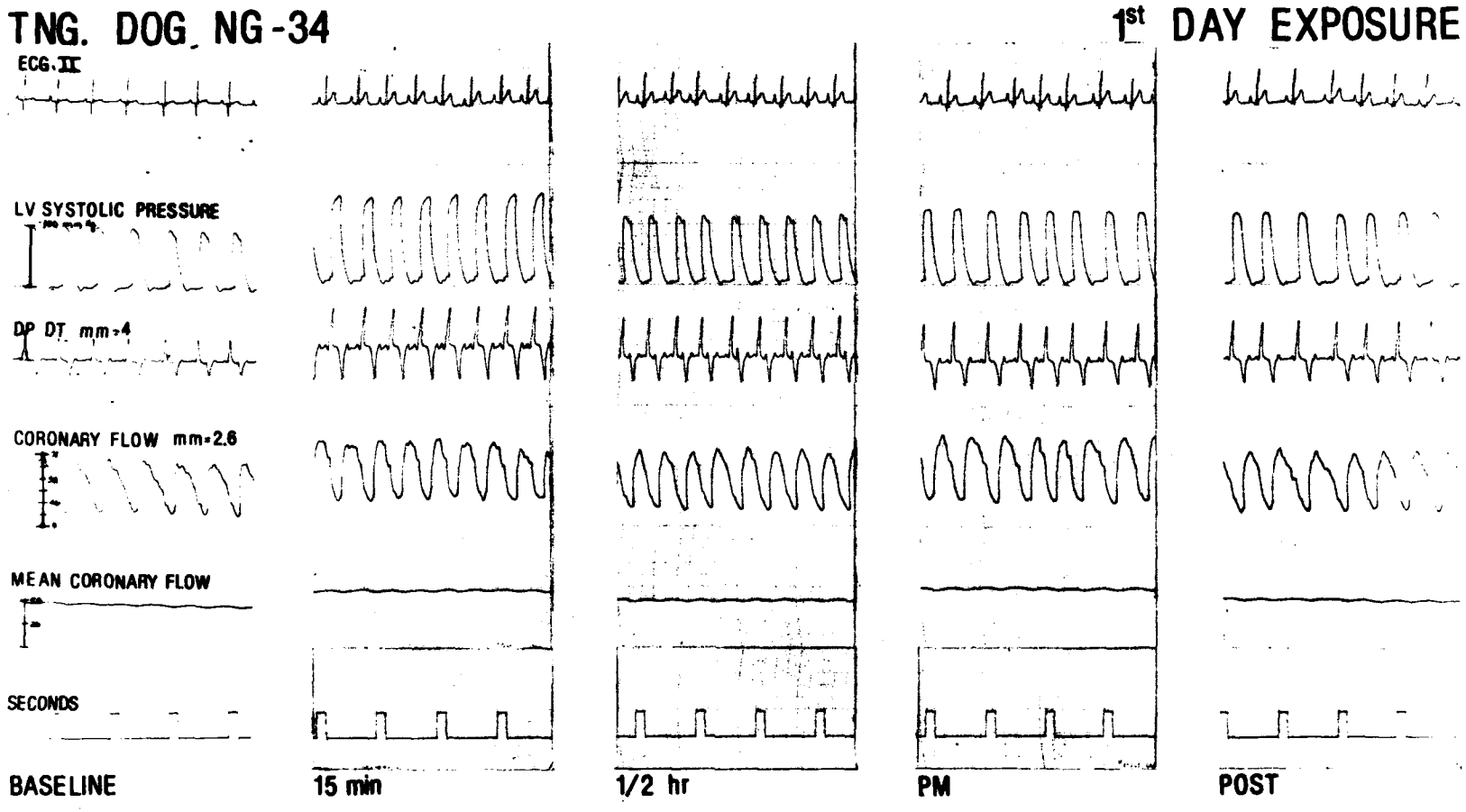


FIGURE 9 REPRESENTATIVE DATA ON ECG, LEFT VENTRICULAR PRESSURE, AND CORONARY BLOOD FLOW IN DO TREATMENT WITH TNG AND RECOVERY

(a) First day of first exposure series.

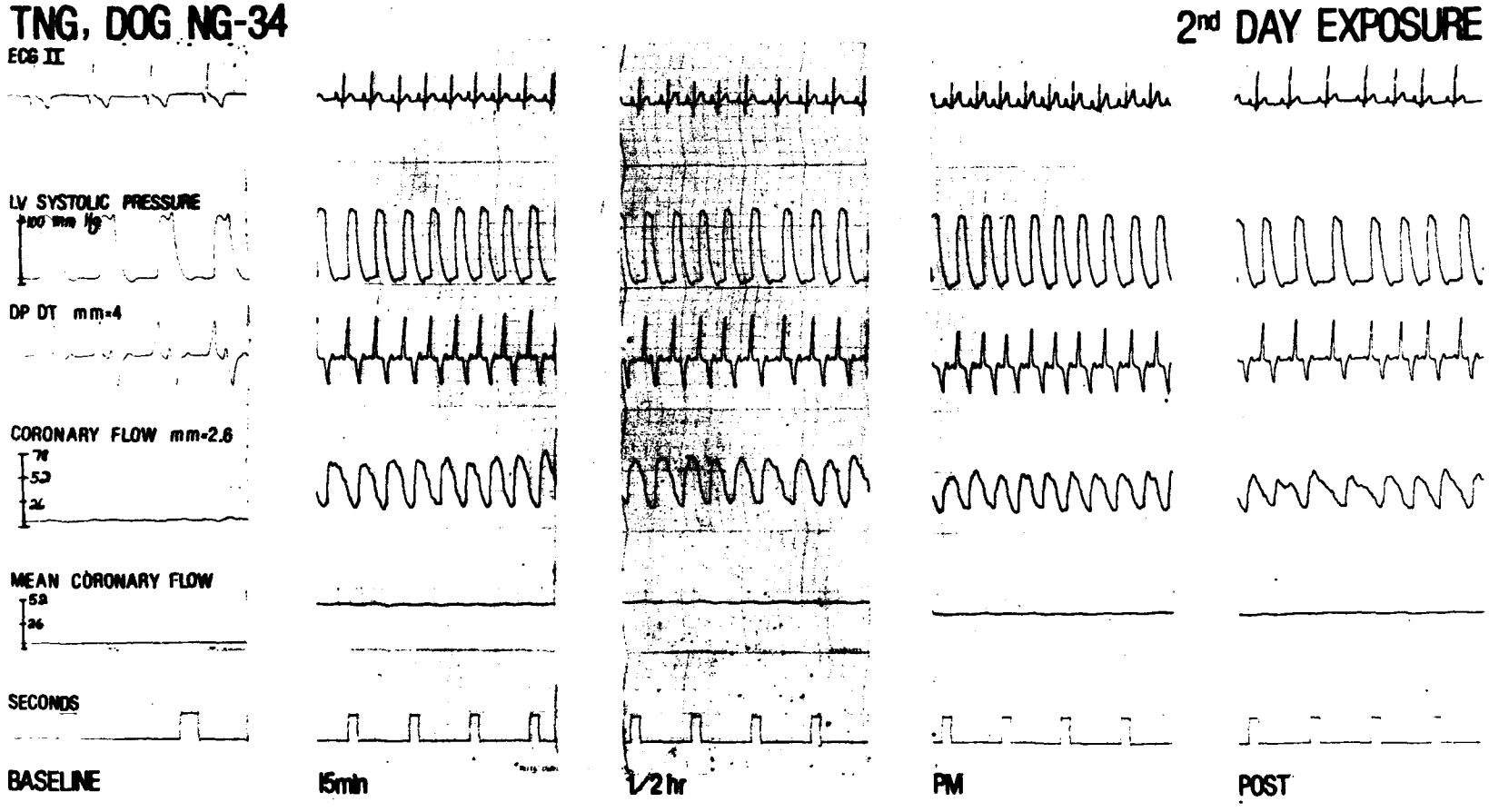


FIGURE 9(b) SECOND DAY OF FIRST EXPOSURE SERIES

TNG, DOG NG-34

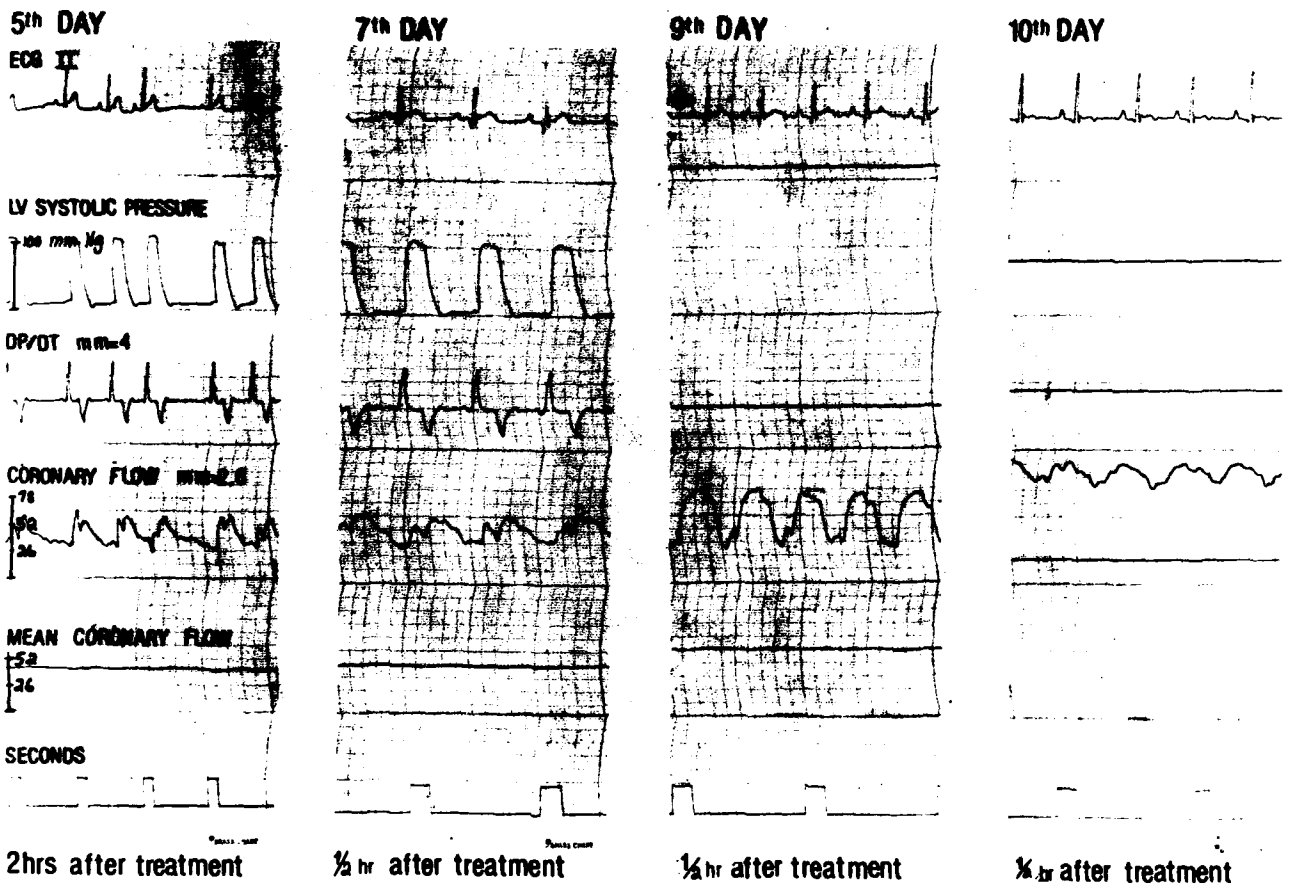


FIGURE 9(c) FIRST EXPOSURE SERIES, DAYS 5, 7, 9 AND 10

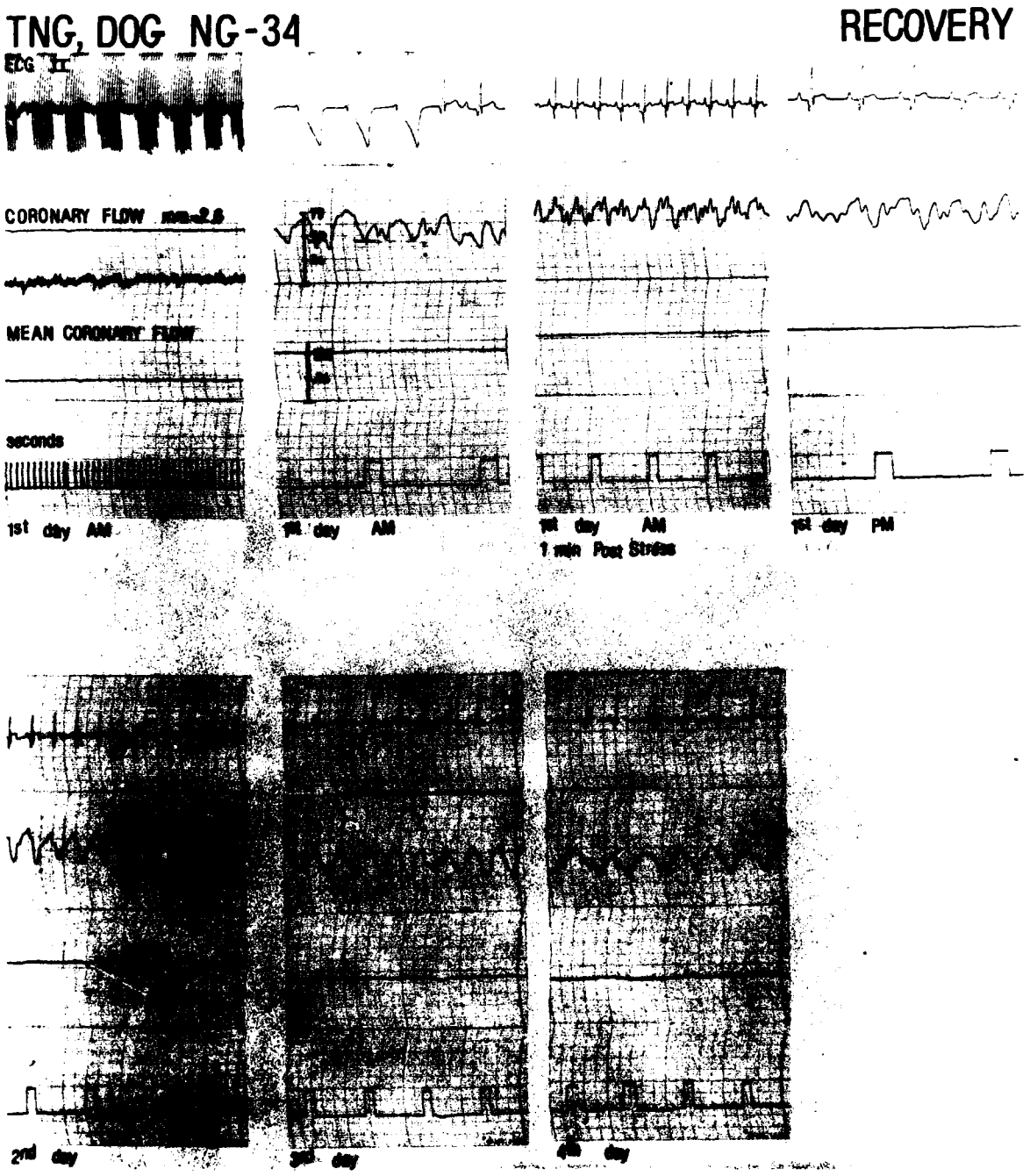


FIGURE 9(d) RECOVERY DAYS, FIRST SERIES

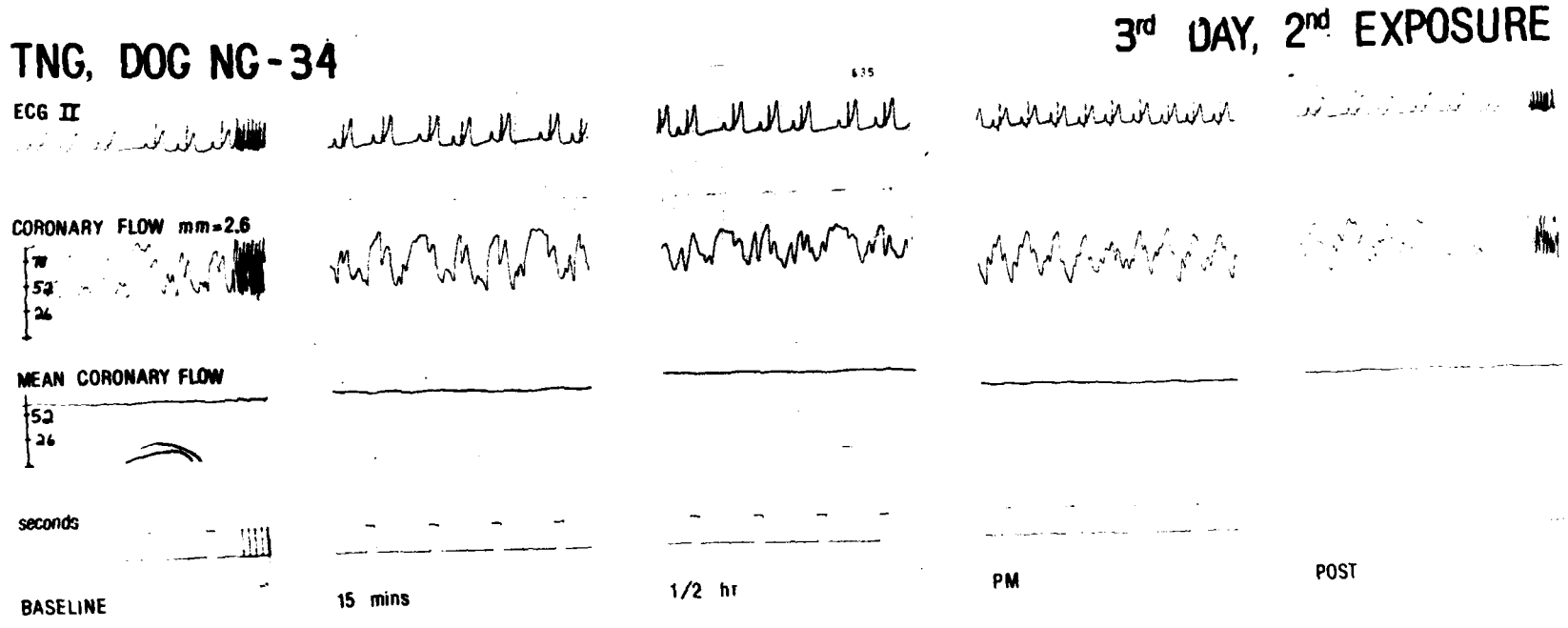


FIGURE 9(e) THIRD DAY OF SECOND EXPOSURE SERIES

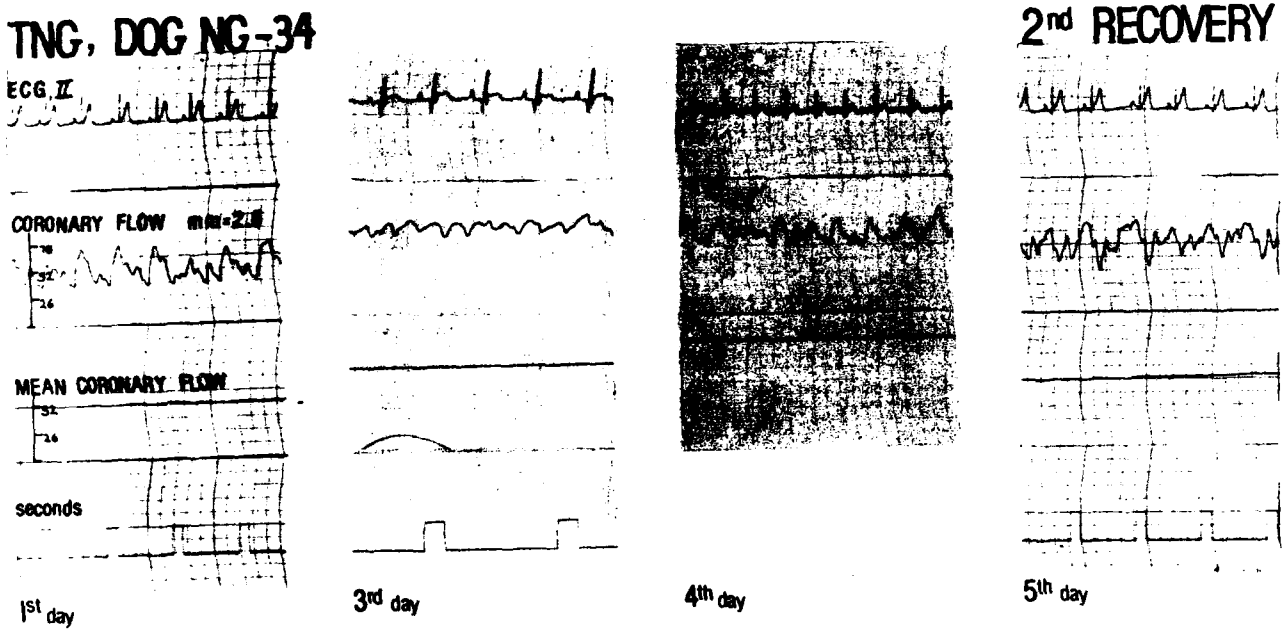


FIGURE 9(f) RECOVERY DAYS, SECOND SERIES

Table 9

LEFT VENTRICULAR PRESSURE, HEART RATE, AND CORONARY FLOW IN DOG NG-34 DURING AND AFTER PERCUTANEOUS EXPOSURE TO 10 g OF 10% NITROGLYCERIN ON LACTOSE

		1st Exposure Series		2nd Exposure Series	
Treatment Day	Time of Reading	Heart Rate	Mean Coronary Artery Flow	Heart Rate	Mean Coronary Artery Flow
1	Baseline	90	50	90	--
	15 min	120	44	75	57
	30 min	120	52	90	59
	Afternoon	90	62	75	52
	Post-exposure	90	52	135	54
2	Baseline	--	--	125	--
	15 min	120	50	90	76
	30 min	120	52	90	73
	Afternoon	135	41	--	--
	Post-exposure	90	41	--	--
3	Baseline	135	50	90	75
	15 min	120	52	90	73
	30 min	135	50	75	78
	Afternoon	75	48	105	--
	Post-exposure	75	50	90	--
4	Baseline	90	43	90	65
	15 min	75	41	90	42
	30 min	120	52	75	--
	Afternoon	120	--	90	60
	Post-exposure	120	50	75	60
5	Baseline	90	44	105	65
	15 min	90	50	90	--
	30 min	90	44	105	26
	Afternoon	105	47	105	65
	Post-exposure	90	44	--	--
6	Baseline	105	52	120	37
	15 min	90	49	120	42
	30 min	60	37	105	42
	Afternoon	75	37	120	40
	Post-exposure	75	37	105	--

(Continued)

Table 9 (Concluded)

Treatment Day	Time of Reading	1st Exposure Series Heart Rate	1st Exposure Series Mean Coronary Artery Flow	2nd Exposure Series Heart Rate	2nd Exposure Series Mean Coronary Artery Flow
7	Baseline	105	52	120	42
	15 min	90	57	120	--
	30 min	75	50	120	--
	Afternoon	74	40	120	--
	Post-exposure	75	35	105	--
8	Baseline	90	45	90	--
	15 min	125	52	90	--
	30 min	90	54	75	--
	Afternoon	75	52	105	42
	Post-exposure	75	37	90	64
9	Baseline	90	57	90	--
	15 min	90	40	90	--
	30 min	105	50	90	--
	Afternoon	125	60	90	--
	Post-exposure	125	54	105	--
10	Baseline	135	--	90	--
	15 min	120	--	90	--
	30 min	120	--	75	--
	Afternoon	105	--	90	--
	Post-exposure	120	--	90	--
1 Recovery		105	--	90	52
		105	74		
		120	62		
		105	74		
2 Recovery		105	60	90	--
		105	60		
3 Recovery		105	60	105	--
		105	53		
		105	--		
		135	62		
4 Recovery		75	54	75	--
		90	57		
		90	39		

Each daily reading, in order, is baseline, 15 min after treatment began, 30 min after treatment began, 4-6 hr after treatment began, and 30 min after TNG was removed.

Table 10

LEFT VENTRICULAR PRESSURE AND HEART RATE
IN DOG NG-35 DURING AND AFTER PERCUTANEOUS EXPOSURE
TO 10 g OF 10% NITROGLYCERIN ON LACTOSE

	First Series of Exposures		Second Series of Exposures	
Exposure Day	Heart Rate	Left Ventricular Pressure	Heart Rate	Left Ventricular Pressure
1	70	60	72	48
	60	64	78	52
	70	52	72	48
	66	--	66	--
2	108	92	60	48
	72	88	72	48
	90	88	72	40
	84	--	60	40
3	90	--	66	--
	72	92	54	--
	72	88	60	--
	96	88	54	--
4	90	100	66	48
	84	56	54	48
	96	56	66	48
	84	39	66	48
5	70	60	72	48
	90	60	48	44
	84	52	72	48
	78	60	54	48
6	78	60	78	48
	70	60	54	44
	120	52	78	44
	108	60	72	48
7	80	48	60	44
	70	48	60	40
	70	52	66	44
	--	--	84	52

(Continued)

Table 10 (Concluded)

Exposure Day	First Series of Exposures		Second Series of Exposures	
	Heart Rate	Left Ventricular Pressure	Heart Rate	Left Ventricular Pressure
8	60	48	78	44
	66	48	54	48
	72	48	54	48
	60	44	66	52
9	72	52	90	52
	60	48	72	48
	72	48	66	52
	60	48	78	48
10	--	--	78	52
	90	52	78	44
	108	48	84	76
	84	48	72	--
1 Recovery	90	56	54	52
	66	48	66	52
	78	48	78	56
	84	52	78	56
2 Recovery	66	48	72	56
	96	48	78	52
	70	48	60	56
	66	52	60	56
3 Recovery	60	48	60	52
	78	48	60	48
	84	48	60	48
	66	48	48	52
4 Recovery	84	44	78	52
	96	44	66	52
	72	44	72	48
	--	--	66	48

Left ventricular pressure, in mm Hg
Heart rate, in beats/minute.

Each daily reading, in order, is baseline, one-half hour after treatment began, 4 to 6 hours after treatment began, and one-half hour after the TNG was removed.

Figure 10 shows LVP and ECG tracings during the two 10-day exposures to TNG and the 4-day recovery periods. The increase in LVP on the second day of exposure was very apparent. Otherwise, there is very little of interest except that the systolic pressure was quite low for a dog.

Table 11 shows the resting LVP and heart rate of dog NG-39. There was a decline in ventricular pressure during the first 10-day treatment series, a marked decrease during the 4-day recovery period, and a slight decrease during the second treatment series, resulting in an overall decrease from the beginning to the end of the study. Studies with other instrumented dogs have shown that the baseline status usually does not change after 2 to 4 days in the laboratory in the absence of drug treatment, and therefore this is likely not to be a training effect. While this observation looks suspiciously like an artifact, there is also a gradual decline in heart rate throughout the study, lending credibility to the observed pressure decrease. This also correlates well with the decrease in spontaneous activity observed in the dog each day.

On a day-by-day basis, the first half hour of treatment generally produced a decrease in heart rate. The pressure usually remained constant or dropped during this time, but on Days 3, 5, 6, 7, and 8 of the first treatment series there was a notable rise in pressure over baseline, even though the heart rate dropped on Days 3, 7, and 8. This might suggest that peripheral resistance (vasoconstriction?) might be increasing. On the other hand, the gradual pressure drop throughout the study might suggest a gradual peripheral vasodilation that is not being compensated for by an increase in heart rate. Unfortunately, the power supply failed just at the end of the second exposure series, so no more data could be obtained. However, it appears that the pressure really decreased starting with the end of the second recovery day following the first exposure series.

Table 12 presents the stress data during and after the first exposure series. On treatment days, the dogs were exercised while being treated. The exercise was a 2-minute trot on the treadmill, unless other times are specified. The data were collected at the post-

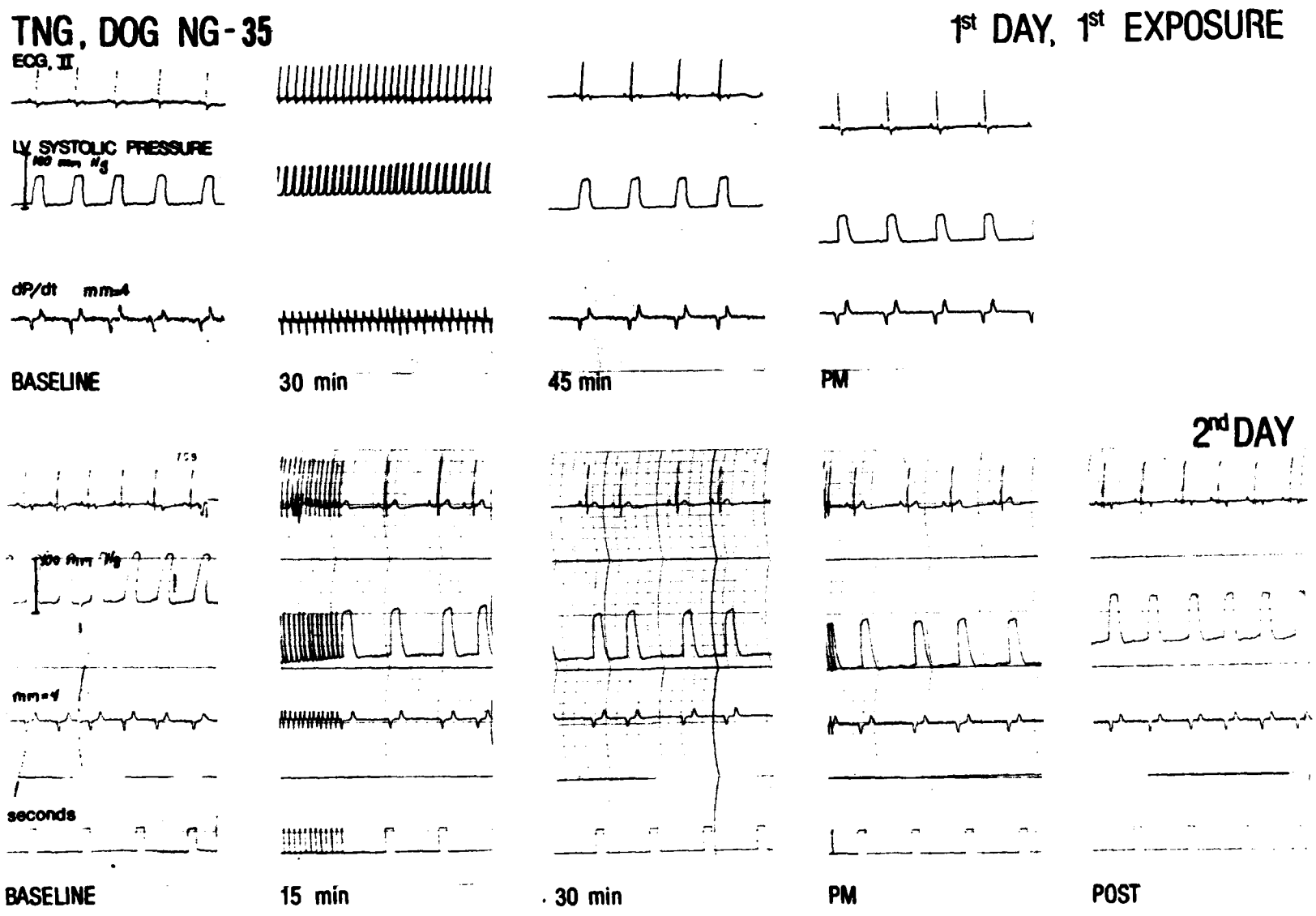


FIGURE 10 REPRESENTATIVE DATA ON ECG AND LVP IN DOG NG-35 DURING TREATMENT WITH TNG AND RECOVERY
(a) First and second days of first exposure series.

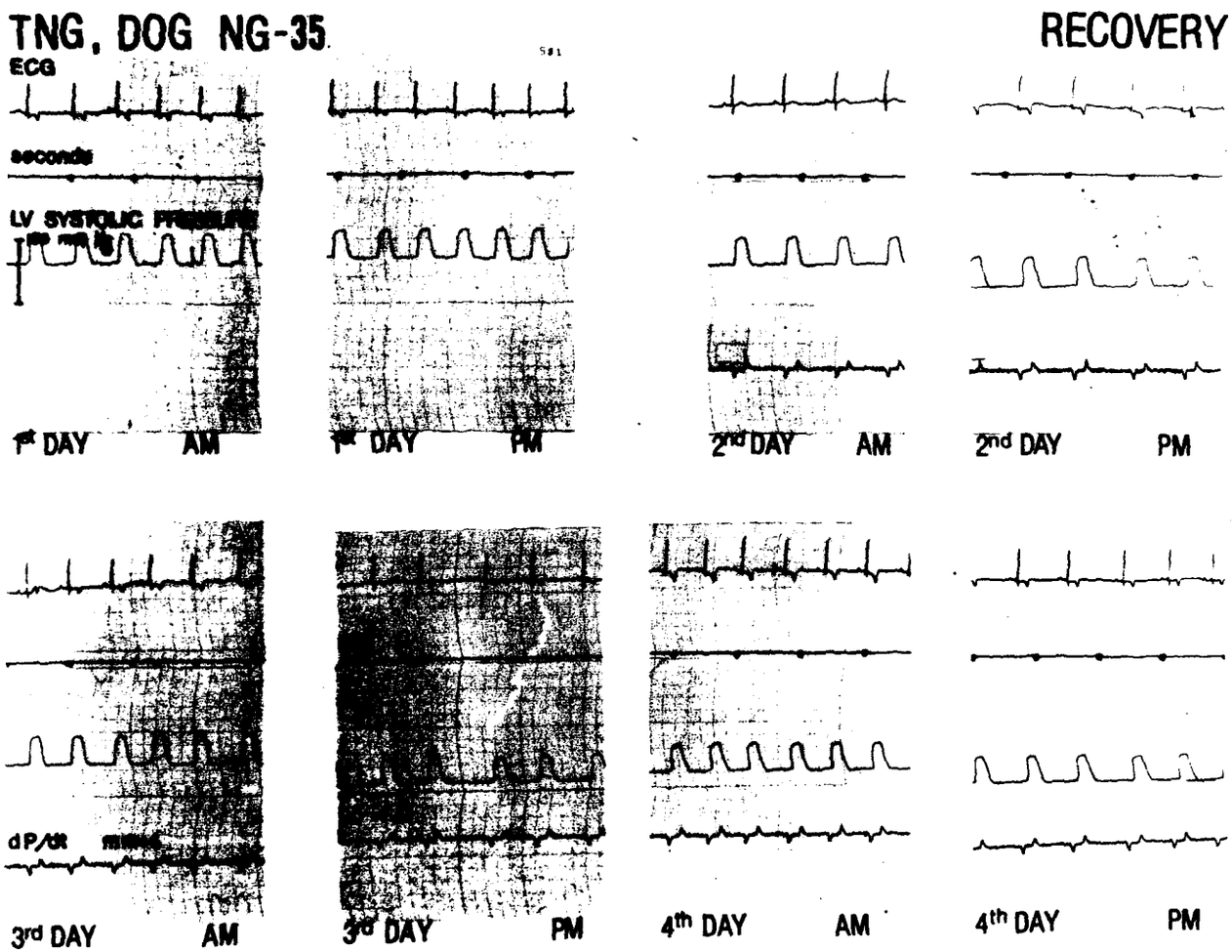


FIGURE 10(b) RECOVERY DAYS, *FIRST SERIES*

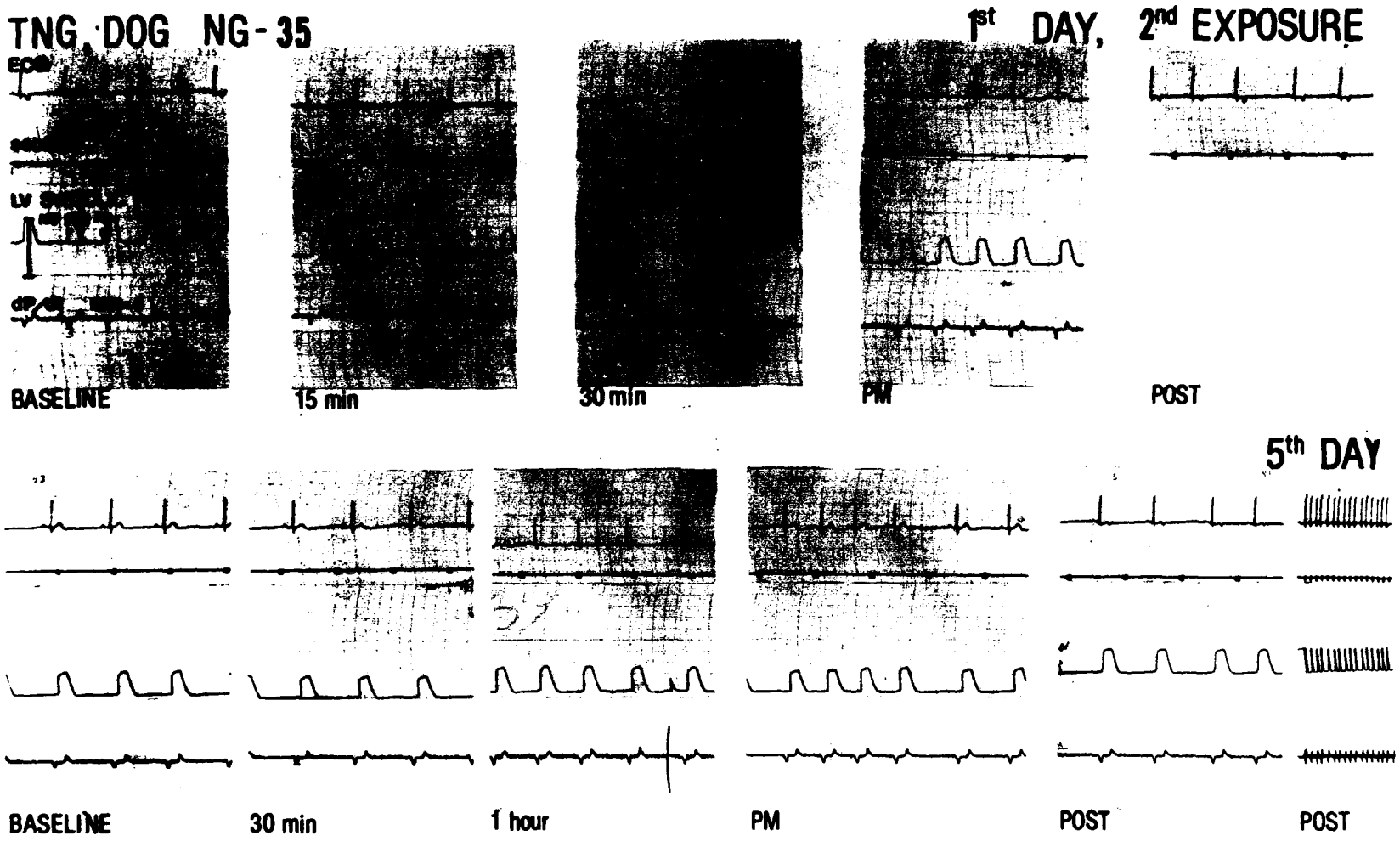


FIGURE 10(c) FIRST AND FIFTH DAYS, SECOND EXPOSURE SERIES

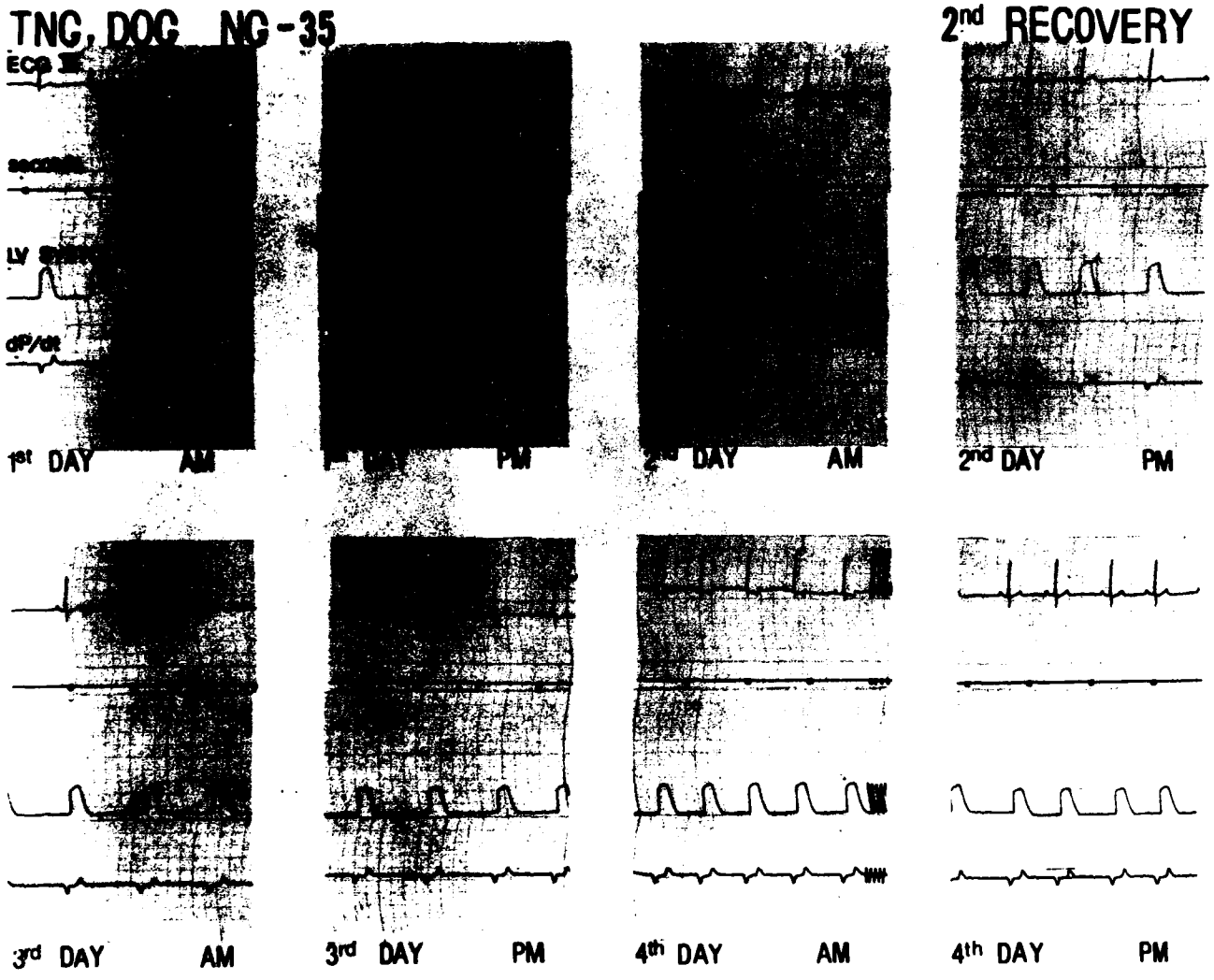


FIGURE 10(d) RECOVERY DAYS, SECOND SERIES

Table 11

RESTING LEFT VENTRICULAR PRESSURE AND HEART RATE OF DOG NG-39 DURING TWO 10-DAY EXPOSURES TO 10 g OF 10% TNG ON LACTOSE AND DURING ONE 4-DAY RECOVERY PERIOD IN BETWEEN EXPOSURE SERIES

Treatment Day	First Exposure LVP (mm Hg)	First Exposure HR (beats/min)	Second Exposure LVP (mm Hg)	Second Exposure HR (beats/min)
1	96	132	36	132
	80	90	36	60
	84	108	32	96
	92	102	32	108
2	88	114	36	84
	84	114	36	84
	--	--	28	66
	--	--	32	66
3	68	126	48	108
	76	96	32	60
	72	126	36	72
	80	102	32	72
4	68	120	36	108
	64	102	32	72
	64	84	36	60
	72	84	28	60
5	52	102	32	90
	68	108	32	72
	88	126	24	66
	72	114	28	102
6	60	90	32	72
	68	102	29	54
	52	--	32	78
	68	78	36	102
7	52	114	32	72
	64	96	24	60
	72	168	32	66
	60	126	32	102
8	52	96	32	102
	68	90	24	54
	64	78	28	54
	--	--	40	108

Table 11(concluded)

Treatment Day	First Exposure LVP (mm Hg)	First Exposure HR (beats/min)	Second Exposure LVP (mm Hg)	Second Exposure HR (beats/min)
9	64	132	32	90
	52	126	24	60
	60	96	28	78
	64	150	28	66
10	72	144	24	72
	64	108	24	54
	64	138	--	66
	60	114	--	96
Recovery				
1	60	138	EF*	120
	80	132		66
	60	132		78
2	60	126		78
	64	96		78
	44	72		84
3	56	126		90
	44	86		78
	52	90		84
4	40	72		
	48	84		
	40	90		

*Electronics failure.

Each daily reading, in order, is baseline, one-half hour after treatment began, 4 to 6 hours after treatment began, and one-half hour after TNG was removed.

Table 12

LEFT VENTRICULAR PRESSURE AND HEART RATE OF DOG NG-39 EXERCISED ON A TREADMILL DURING DAYS 7-10 OF EXPOSURE TO 1.0 g OF 10% TNG ON LACTOSE AND DURING THE 4-DAY RECOVERY PERIOD

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
7	1 min after exercise	76	138
	2 min after exercise	76	138
	3 min after exercise	72	126
	4 min after exercise	68	126
	7 min after exercise	64	114
8	Morning:		
	During stress	104	150
	1 min after exercise	76	150
	2 min after exercise	72	138
	3 min after exercise	64	108
	5 min after exercise	64	96
	Afternoon:		
	1 min after exercise	80	162
	3 min after exercise	80	138
	5 min after exercise	72	126
9	Morning:		
	During exercise	88	--
	1 min after exercise	64	126
	2 min after exercise	64	120
	4 min after exercise	68	126
	5 min after exercise	60	120
	Afternoon:		
	1 min after exercise	64	120
	3 min after exercise	64	138
	4 min after exercise	56	108
	5 min after exercise	60	132

(Continued)

Table 12 (Continued)

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
10	Morning:		
	During exercise	64	96
	1 min after exercise	60	108
	2 min after exercise	60	102
	4 min after exercise	64	114
1 Recovery	Morning:		
	During 1-min exercise	64	--
	During 3-min exercise	56	--
	During 5-min exercise	56	--
	1 min after exercise	52	108
	2 min after exercise	52	96
	3 min after exercise	52	96
	Midday:		
	2-min exercise	72	--
	1 min after exercise	64	102
	3 min after exercise	60	114
	5 min after exercise	60	108
	Afternoon:		
	1 min after exercise	44	90
	3 min after exercise	52	96
	5 min after exercise	52	84
2 Recovery	Morning exercise	56	102
	1 min after exercise	48	108
	2 min after exercise	52	96
	5 min after exercise	60	126
	Midday exercise	64	132
	2 min after exercise	56	96
	4 min after exercise	56	96
	6 min after exercise	56	108
	Afternoon exercise	60	138
	1 min after exercise	48	90
	3 min after exercise	48	90
	5 min after exercise	48	108

(Continued)

Table 12 (Concluded)

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
3 Recovery	Morning exercise	48	132
	1 min after exercise	40	84
	2 min after exercise	44	96
	5 min after exercise	44	102
	Afternoon exercise	52	--
	1 min after exercise	40	84
	3 min after exercise	40	78
	6 min after exercise	36	78
4 Recovery	Morning exercise	44	132
	1 min after exercise	36	90
	3 min after exercise	36	84
	5 min after exercise	44	78

exercise times indicated. In general, the recovery from exercise--both in pressure and heart rate--was rapid, and within a few minutes the values were approximately equivalent to the resting values (shown in Table 11). It is interesting to note the pressure-heart rate adjustments that took place 4 to 5 minutes after exercise. There was often a slight increase in LVP and/or heart rate after the initial decrease from the exercise-induced changes in pressure and heart rate.

Table 13 shows the exercise data collected daily during the second exposure series. The LVP remained low, even during exercise; hence, there was little change during the period after stress. Exercise adjustments seemed to be mostly in heart rate. (Possibly the continued low LVP, even when the heart rate increased, indicates a continued vasodilation.) It is unfortunate that the battery failed at the end of the second exposure period because it would have been interesting to follow this dog for several days in the recovery phase.

Figure 11 shows representative data on ECG and LVP collected during the treatment period and recovery for dog NG-39. Note that the T-wave of the ECG became increasingly more inverted as the experiment progressed and did not recover at all during the recovery period.

Table 14 shows the resting heart rate and the pulsatile and mean coronary flow of dog NG-41 during the exposure-recovery sequence of treatments. Generally, the heart rate decreased considerably during 15 to 30 minutes after the start of treatment, but had increased again by the end of the treatment and at the post-exposure readings. However, this was also the general pattern on all the recovery days except that it was not so apparent on Days 1 and 3 of the second recovery series.

On the first five days of the first exposure series, mean coronary flow increased or remained the same as baseline during the first 15 and 30 minutes of treatment. On the first and second treatment days, mean coronary flow increased 4 to 6 hours after treatment began. This assessment cannot be made for Days 6, 8, and 9 and the first three recovery days in the first exposure series due to temporary electronics problems (noise, interference, etc.). In general, on all other

Table 13

EXERCISE DATA DURING SECOND EXPOSURE SERIES
OF DOG NG-39 TO 1 g OF 10% TNG ON LACTOSE

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
1	Morning:		
	1 min after exercise	36	--
	2 min after exercise	36	120
	3 min after exercise	40	102
	5 min after exercise	44	120
	Afternoon:		
	3-min exercise	36	132
	1 min after exercise	40	--
	2 min after exercise	36	102
	4 min after exercise	36	96
2	Morning:		
	2-min exercise	36	--
	1 min after exercise	36	84
	3 min after exercise	32	72
	5 min after exercise	32	72
	Afternoon:		
	2-min exercise	44	132
	1 min after exercise	40	--
	3 min after exercise	32	72
	5 min after exercise	32	72
3	Morning:		
	1-min exercise	40	--
	2 min after exercise	32	72
	3 min after exercise	32	66
	4 min after exercise	32	78
	Afternoon:		
	2-min exercise	38	--
	1 min after exercise	32	--
	2 min after exercise	32	84

(Continued)

Table 13 (Continued)

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
4	Morning:		
	1 min after exercise	28	84
	2 min after exercise	28	84
	Afternoon:		
	1 min after exercise	32	90
	3 min after exercise	32	66
	5 min after exercise	28	54
5	Morning:		
	1 min after exercise	32	78
	2 min after exercise	32	72
		28	78
	Afternoon:		
	1 min after exercise	24	102
	2 min after exercise	28	60
	5 min after exercise	28	72
6	During 2-min exercise	32	--
	2 min after exercise	28	78
	4 min after exercise	28	72
	5 min after exercise	28	60
7	Morning:		
	Exercise	28	--
	1 min after exercise	28	78
	2 min after exercise	24	72
	4 min after exercise	28	72
	Afternoon:		
	2-min exercise	32	--
	1 min after exercise	36	--
	2 min after exercise	28	96
	4 min after exercise	28	72

(Continued)

Table 13 (Concluded)

Treatment Day	Time of Reading	LVP (mm Hg)	HR (beats/min)
9	Morning:		
	2-min exercise	28	--
	2 min after exercise	20	96
	3 min after exercise	20	60
	4 min after exercise	20	54
	5 min after exercise	20	60
	Afternoon:		
	2-min exercise	20	--
	2 min after exercise	20	60
	3 min after exercise	24	54
	4 min after exercise	20	54
	5 min after exercise	20	54

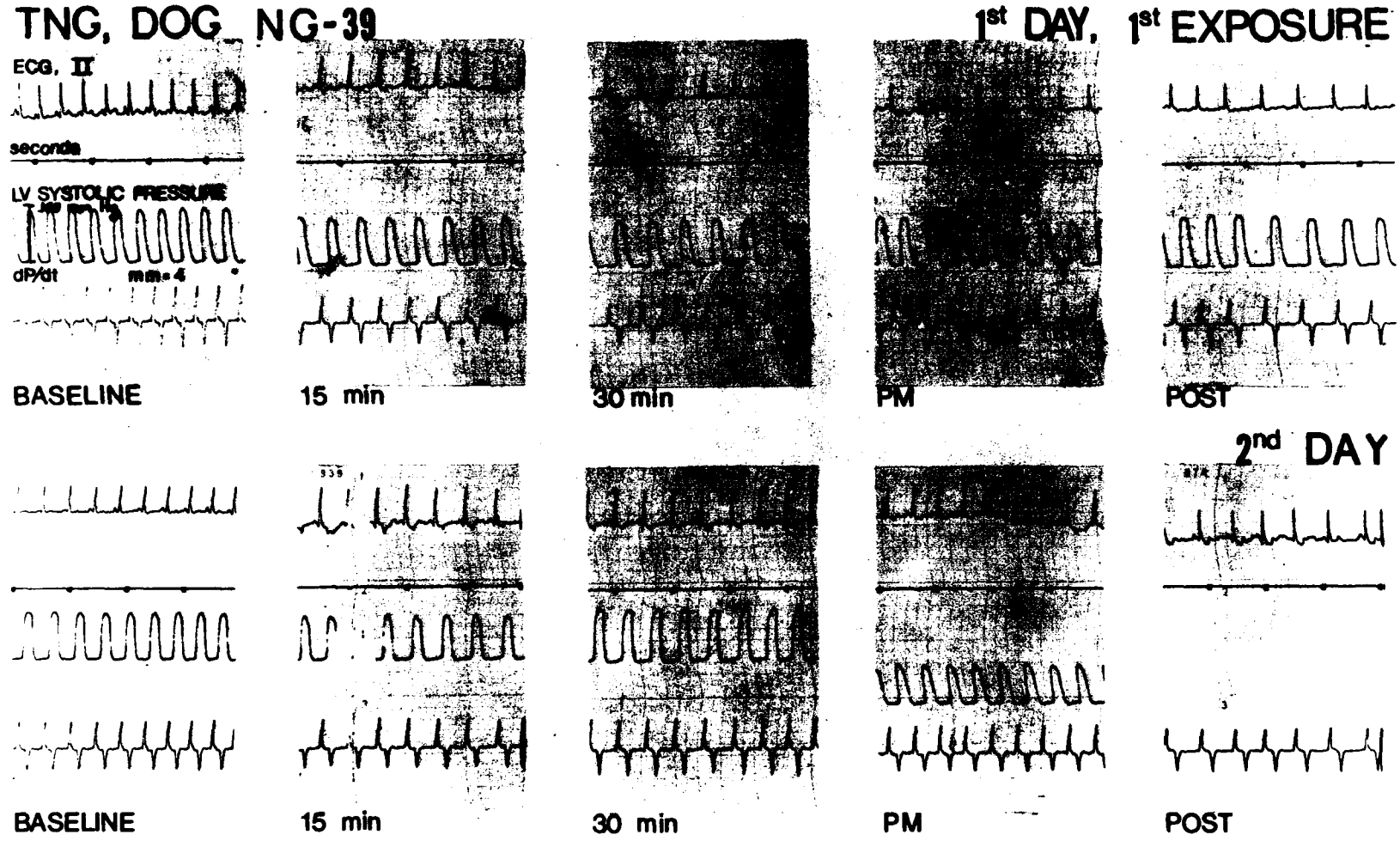


FIGURE 11 REPRESENTATIVE DATA ON ECG AND LVP DURING TREATMENT WITH TNG AND RECOVERY IN DOG NG-39

(a) First and second days of first exposure series.

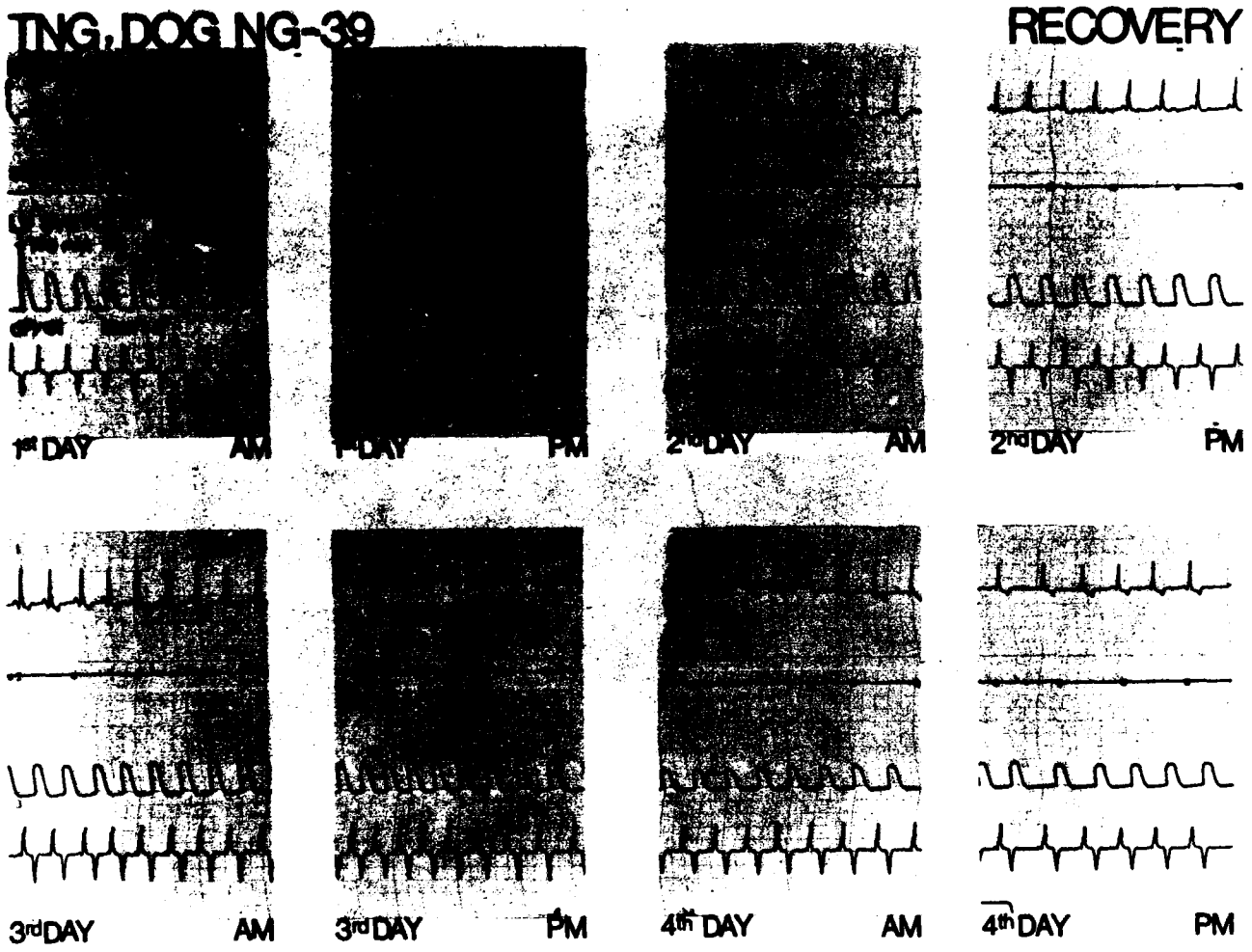


FIGURE 11(b) RECOVERY DAYS, FIRST SERIES

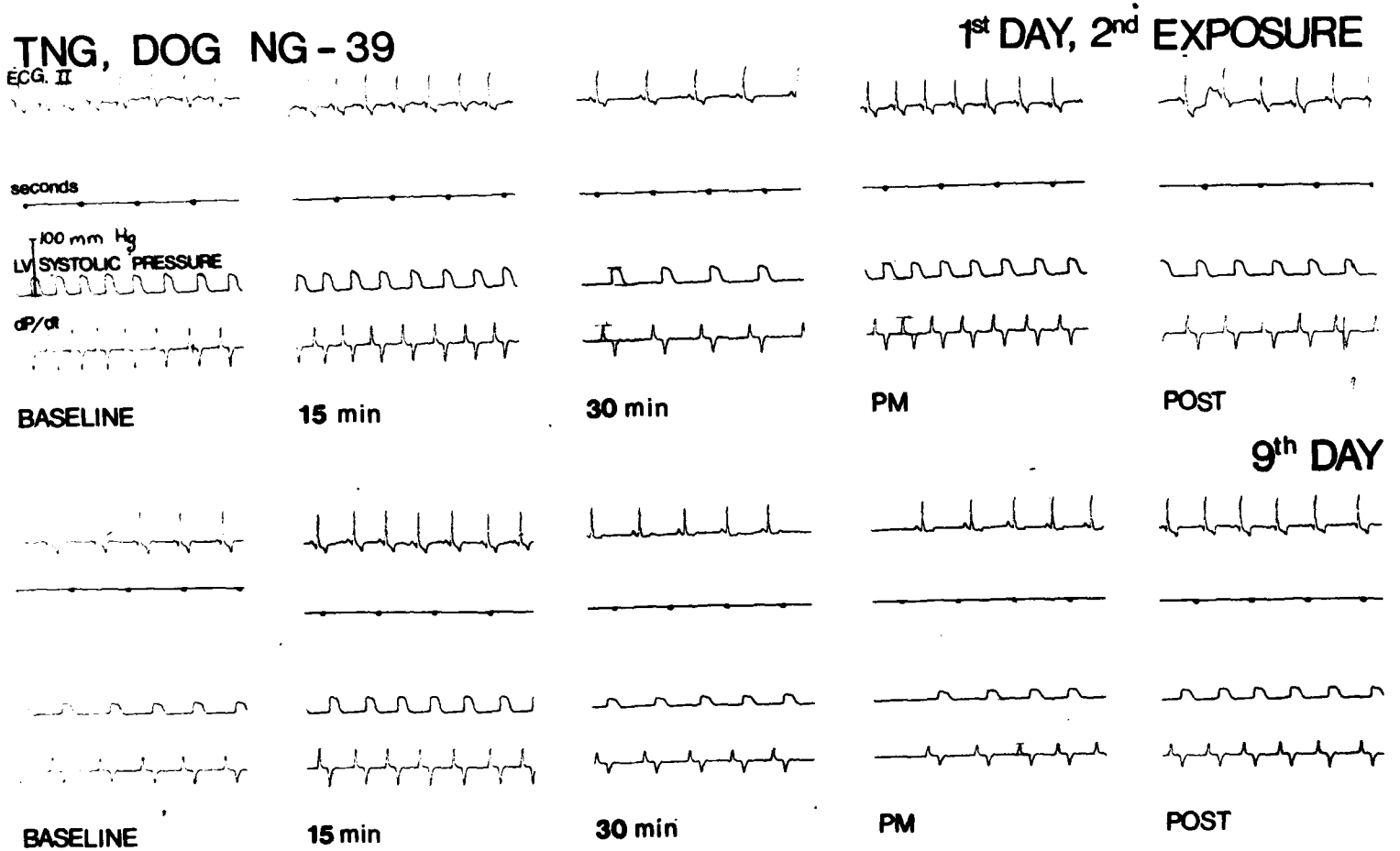


FIGURE 11(c) FIRST AND NINTH DAYS, SECOND EXPOSURE SERIES

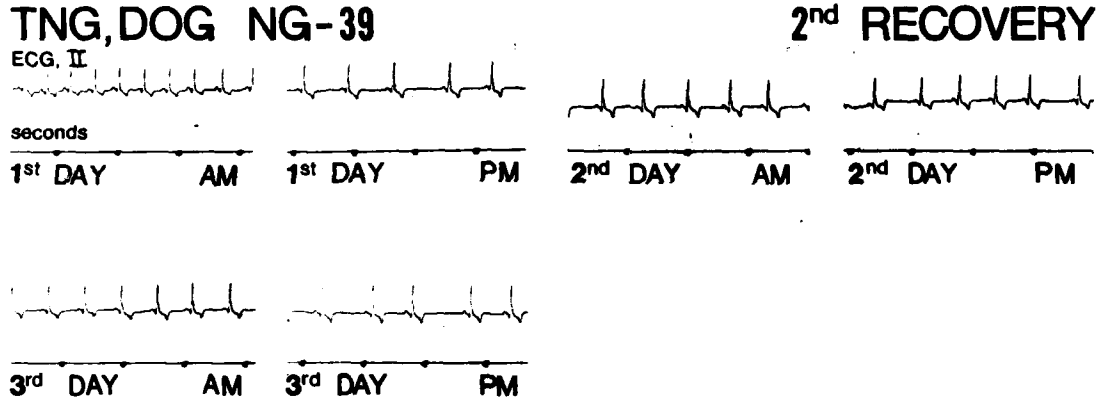


FIGURE 11(d) RECOVERY DAYS, SECOND SERIES

Table 14

RESTING CORONARY FLOW AND HEART RATE OF DOG NG-41 DURING TWO 10-DAY EXPOSURES TO 10 g OF 10% TNG ON LACTOSE AND TWO 4-DAY RECOVERY PERIODS

	First Exposure			Second Exposure		
Treatment Day	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
1	109/47	39	108	78/13	60	120
	101/5	50	90	62/23	60	66
	99/5	57	90	68/5	52	78
	106/0	52	114	80/9	70	108
	104/0	62	84	78/8	68	84
2	106/47	54	96	78/5	60	114
	88/0	57	90	52/10	42	72
	91/8	60	84	67/8	52	96
	96/5	60	90	78/10	65	84
	78/13	60	90	73/8	57	96
3	78/34	54	114	78/23	65	108
	73/28	54	96	60/80	52	60
	78/18	62	84	73/8	54	78
	89/16	57	102	65/5	44	84
	83/23	57	102	73/16	54	80
4	88/44	52	126	73/16	57	80
	83/34	57	108	55/5	47	66
	80/18	57	108	60/8	36	66
	78/24	62	102	65/8	50	66
	78/26	62	102	76/5	36	114
5	83/42	60	132	78/31	65	96
	78/24	65	96	54/0	47	72
	80/32	65	96	55/3	39	66
	60/18	65	90	70/10	55	84
	65/8	57	102	78/13	67	96
6	73/23	65	114	76/13	57	90
	EF*	EF	78	52/0	39	72
	EF	EF	96	54/13	42	66
	54/8	57	90	65/5	57	84
	70/8	65	102	60/5	44	84

(Continued)

* EF = Electronics failure (noise, electronic interferences, etc.

Table 14 (Continued)

Treatment Day	First Exposure: Coronary Artery Flow (cm/sec)	First Exposure: Mean Coronary Artery Flow (cm/sec)	First Exposure: Heart Rate (beats/min)	Second Exposure: Coronary Artery Flow (cm/sec)	Second Exposure: Mean Coronary Artery Flow (cm/sec)	Second Exposure: Heart Rate (beats/min)
7	62/16	60	96	65/18	52	84
	47/0	39	78	57/3	47	66
	50/8	42	72	62/3	50	66
	54/5	50	84	65/5	52	72
	62/10	52	90	101/13	54	84
8	EF	EF	108	112/39	68	114
	54/18	54	102	80/8	44	72
	73/18	57	84	94/5	42	66
	81/18	52	84	104/16	57	66
	81/18	68	84	85/10	65	96
9	75/23	62	102	86/26	65	102
	EF	EF	78	57/5	29	66
	70/10	62	84	57/10	34	60
	73/3	52	72	80/5	52	66
	78/13	54	78	91/16	68	102
10	65/10	42	96	78/8	52	96
	57/3	39	84	73/21	52	78
	54/8	49	84	59/3	39	72
	78/3	EF	96	76/8	52	90
	75/5	EF	96	91/8	65	96
1 Recovery	78/6	EF	114	72/8	50	78
	62/18	EF	72	57/13	42	72
	57/13	EF	72	62/13	50	84
	60/13	EF	72	75/10	52	84
	65/23	EF	96			
	62/18	EF	80			
	73/26	EF	114			
2 Recovery	83/29	EF	102	101/18	70	102
	73/13	52	78	70/0	39	66
	62/10	34	80	70/0	39	72
	57/21	31	84	72/5	54	72
	62/21	42	80	65/10	47	84
3 Recovery	EF	EF	102	73/5	39	78
	EF	EF	72	65/0	28	66
	EF	EF	78	68/10	52	78
	70/26	57		65/0	36	72
	57/10	42	102	63/10	41	78

(Continued)

Table 14 (Concluded)

Treatment Day	First Exposure			Second Exposure		
	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
4	70/30	52	102	83/10	52	102
Recovery	65/23	56	80	78/18	52	84
	75/18	62	80	65/16	39	84
	62/13	44	102	73/18	39	78
	49/8	39	80			

Each daily reading, in order, is baseline, 15 minutes after treatment began, 30 minutes after treatment began, 4-6 hours after treatment began, and one-half hour after TNG was removed.

treatment days in both series of exposures, mean coronary flow decreased upon application of TNG. Changes in these flow rates could be due to changes in ventricular pressure or in coronary artery diameter. Based on these data plus those from another dog (NG-39, Table 11), it appears that the general trend was for the LVP to drop after treatment was initiated. Therefore, we cannot tell whether the coronary flow changes are due to changes in arterial diameter or pressure.

There were changes in the maximum and minimum flow during the course of treatment. These changes did not necessarily correlate with changes in heart rate. The maximum flow had a tendency to decrease after the first day of the first exposure and to stabilize at lower flow rates after a few days. No change was generally noticed during the recovery period or the second exposure series. Minimum flow rates increased on the second through the fifth day and generally stayed at a high rate until the second and third day of recovery from the second exposure series.

Table 15 shows the exercise data collected on dog NG-41 during and after the first exposure series. The data were collected after the dog had trotted on the treadmill for 2 min, unless other times are specified. On Days 6, 7, and 8 the heart rate was slightly higher after exercise than on other days. On Days 5 and 7 the maximum flow rate was the lowest. Mean coronary flow did not show any consistent changes. Mean flow after exercise was not markedly different from resting mean flow rates.

Table 16 shows the results of exercise in dog NG-41 during and after recovery from the second TNG exposure series. Again, no consistent pattern was evident. The maximum flow after exercise was often slightly lower than the resting maximum flow. However, the flow and heart rates after exercise were generally close to those taken at rest.

Figure 12 shows representative data collected from dog NG-41 during treatment and recovery. Although the pressure transducer failed in this dog, the coronary flow probes functioned excellently throughout the entire treatment and recovery period. In addition, this dog had a normal ECG throughout the entire experiment.

Table 15

CORONARY FLOW RATES IN DOG NG-41 SUBJECTED TO TREADMILL STRESS DURING EXPOSURE AND RECOVERY FROM THE FIRST TNG EXPOSURE SERIES

First Exposure, First Series

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
4	Morning	1 min	86/26	60	108
		2 min	86/26	54	102
		4 min	80/18	57	102
		5 min	80/18	62	96
4	Afternoon	1 min	78/18	65	96
		2 min	104/36	75	108
		4 min	102/39	75	96
4	Post-Exposure	1 min	78/16	62	102
		2 min	76/16	62	102
		3 min	73/18	62	90
		4 min	76/13	62	90
		5 min	76/18	62	84
5	Morning	1 min	54/13	47	96
		2 min	50/10	42	102
		3 min	57/13	48	96
		4 min	65/21	60	102
		5 min	68/18	62	96
5	Afternoon	1 min	54/5	54	84
		2 min	59/13	62	90
		3 min	59/13	62	84
		4 min	58/13	65	90
6	Morning	1 min	62/10	62	120
		2 min	57/10	52	108
		3 min	60/8	52	102
		4 min	50/5	36	102
6	Afternoon	1 min	68/5	62	108
		2 min	68/10	65	108
		3 min	65/8	65	102
		4 min	62/8	60	102
		5 min	62/13	62	108
		1 min			132
		2 min	68/5	58	120
		3 min	65/8	54	102
		4 min	57/8	54	114
		5 min	65/13	56	114

(Continued)

Table 15 (Continued)

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
7	Morning	1 min	52/8	52	114
		2 min	70/3	36	102
		3 min	36/8	36	114
		4 min	EF	EF	90
		5 min	47/3	42	90
7	Afternoon	1 min	62/10	59	114
		2 min	65/8	65	114
		3 min	65/13	57	108
		4 min	68/16	57	114
		5 min	57/10	57	96
8	Morning	1 min	80/10	65	132
		2 min	80/10	65	120
		3 min	73/10	54	108
		4 min	73/18	57	108
		5 min	78/16	56	108
9	Morning	1 min	60/10	42	114
		2 min	68/0	54	102
		3 min	73/16	65	102
		4 min	73/5	65	96
		5 min	68/3	44	60
9	Afternoon	1 min	75/13	54	84
		2 min	75/21	68	90
		3 min	70/13	50	72
		4 min	65/0	57	72
		5 min	70/16	52	102
10	Morning	1 min			108
		2 min	70/10	44	102
		3 min	62/13	50	96
		4 min	60/10	EF	90
		5 min	54/10	EF	90
10	Afternoon	1 min	73/5	EF	114
		2 min	57/0	EF	108
		3 min	76/3	EF	102
		4 min	60/0	EF	84
		5 min	54/0	EF	102

(Continued)

Table 15 (Concluded)

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
1 Recovery	Morning	1 min	68/13	EF	96
		2 min	57/8	EF	102
		4 min	48/10	EF	90
		5 min	44/10	EF	84
1 Recovery	Afternoon	1 min	65/21	EF	114
		2 min	65/10	EF	90
		3 min	50/10	EF	84
		4 min	54/10	EF	84

Table 16

CORONARY FLOW RATES IN DOG NG-41 SUBJECTED TO TREADMILL STRESS DURING EXPOSURE AND RECOVERY FROM THE SECOND TNG EXPOSURE SERIES

Second Exposure, Second Series

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
1	Morning	1 min	73/10	60	120
		2-3 min	65/0	50	108
		4-5 min	59/0	42	102
2	Morning	1 min	65/5	52	102
		2 min	63/5	47	102
		3 min	62/3	52	102
		4 min	54/5	39	96
		5 min	54/5	34	90
2	Afternoon	1 min	65/16	47	90
		2 min	73/15	52	90
		3 min	73/13	65	90
		4 min	65/8	52	84
		5 min	68/0	52	78
3	Morning	1 min	-	-	90
		2 min	6?/16	54	84
		3 min	57/16	52	90
		4 min	60/0	44	72
		5 min	57/10	50	84
4	Morning	2 min	52/5	34	84
		3-4 min	57/10	47	84
		5 min	52/5	34	78
4	Mid-day	1 min	75/8	52	84
		2 min	70/10	49	78
		4-5 min	73/8	57	72
4	Afternoon	1 min	86/13	65	90
		2 min	75/5	49	78
		3-4 min	78/10	62	78
5	Morning	1 min	62/5	57	90
		2 min	52/5	49	84
		3 min	62/8	52	78
		4 min	55/0	44	78
		5 min	55/0	39	72

(Continued)

Table 16 (Continued)

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
5	Afternoon	1 min	65/3	52	78
		2-3 min	52/0	39	78
		5 min	EF	EF	60
6	Morning	2 min	60/5	47	84
		3 min	55/5	47	84
		4 min	47/5	36	72
		5 min	55/5	34	72
6	Afternoon	1 min	68/3	52	102
		2 min	65/5	52	102
		3 min	65/0	44	96
		4 min	70/0	52	96
		5 min	68/13	49	84
7	Morning	1 min	70/18	62	90
		2-3 min	65/8	57	72
		4-5 min	68/5	49	66
8	Morning	1 min	101/39	60	84
		2-3 min	88/18	55	78
		5 min	88/18	44	78
8	Afternoon	1 min	109/34	65	96
		2 min	101/18	65	90
		4 min	101/34	68	90
		5 min	91/18	57	90
9	Morning	1 min	83/26	62	114
		2 min	70/21	55	84
		3 min	78/26	52	78
		4 min	62/15	42	66
		5 min	57/10	34	66
10	Morning	1 min	65/10	39	84
		2 min	70/8	44	84
		3 min	65/5	44	84
		4 min	60/0	36	66
		5 min	65/5	36	84
10	Afternoon	1 min	78/5	49	90
		3 min	70/3	44	84
		5 min	86/5	57	84

(Continued)

Table 16 (Concluded)

Treatment Day	Time of Exercise	Time After Exercise	Coronary Artery Flow (cm/sec)	Mean Coronary Artery Flow (cm/sec)	Heart Rate (beats/min)
1 Recovery	Mid-day	2 min	65/10	36	72
		3 min	68/13	42	72
		4 min	60/5	34	66
		5 min	57/0	18	66
2 Recovery	Morning	1 min	80/8	49	84
		2 min	73/10	52	84
		3 min	60/3	34	78
		4 min	62/10	42	72
		5 min	62/10	34	72
2 Recovery	Afternoon	1 min	67/8	49	90
		2 min	68/5	39	66
		4 min	-	-	66
3 Recovery	Afternoon	1 min	70/5	37	78
		2 min	70/5	44	66
		3 min	62/0	34	60
		4 min	62/8	34	60
4 Recovery	Morning	1 min	81/13	57	84
		2 min	86/13	55	96
		3 min	70/5	47	96
		5 min	65/10	44	96
4 Recovery	Afternoon	1 min	70/5	49	60
		2 min	62/10	49	60
		5 min	39/0	21	54

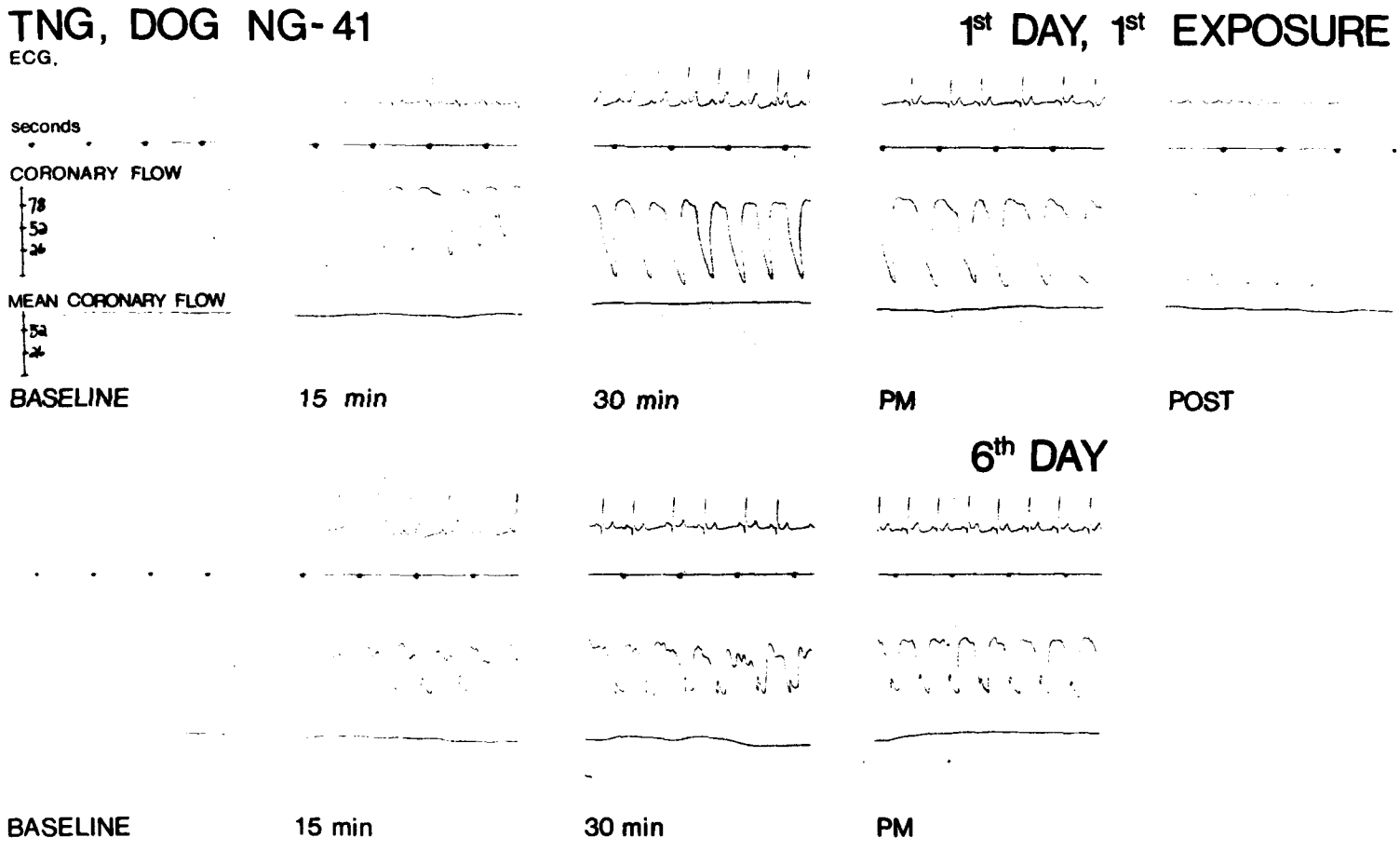


FIGURE 12 REPRESENTATIVE ECG AND CORONARY FLOW DATA COLLECTED FROM DOG NG-41 DURING TREATMENT WITH TNG DURING RECOVERY

(a) First and sixth days, first exposure series.

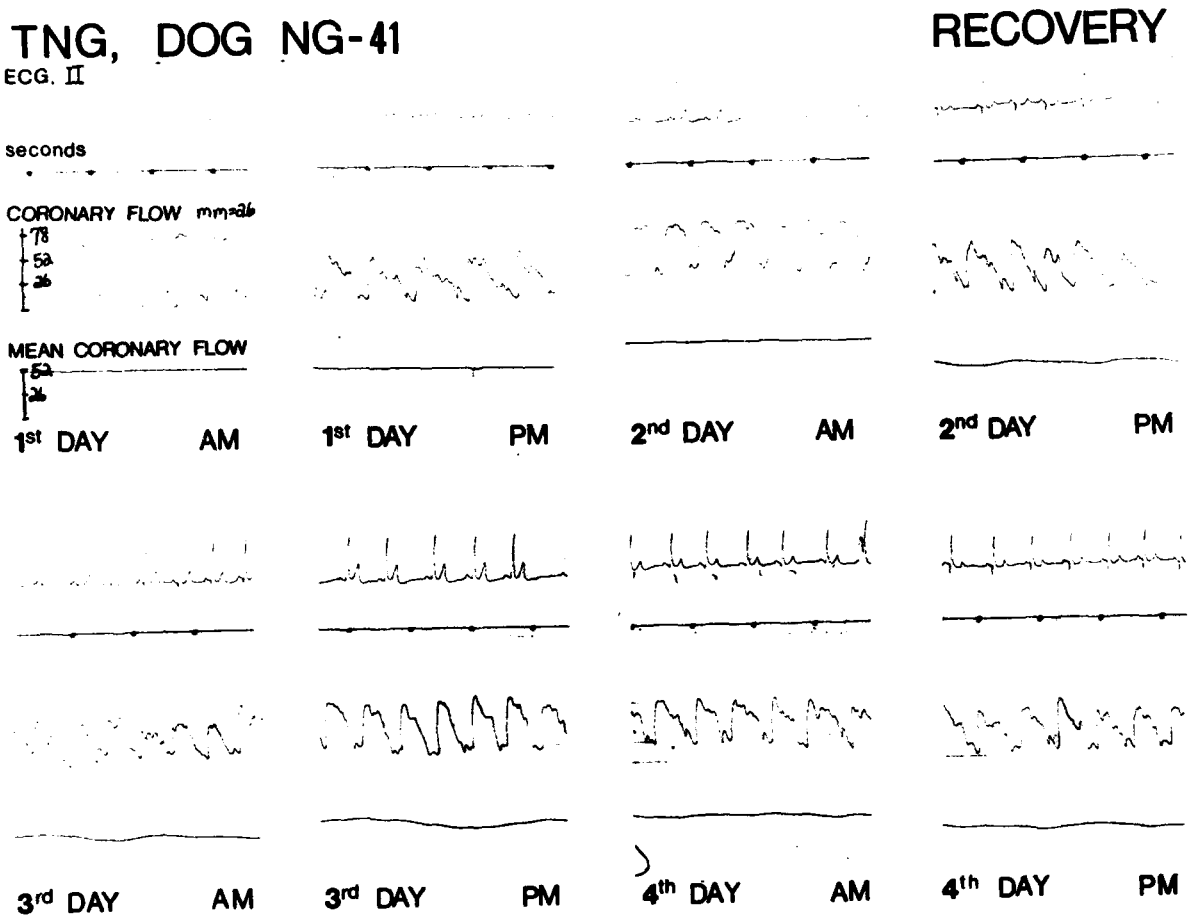


FIGURE 12(b) RECOVERY DAYS, FIRST SERIES

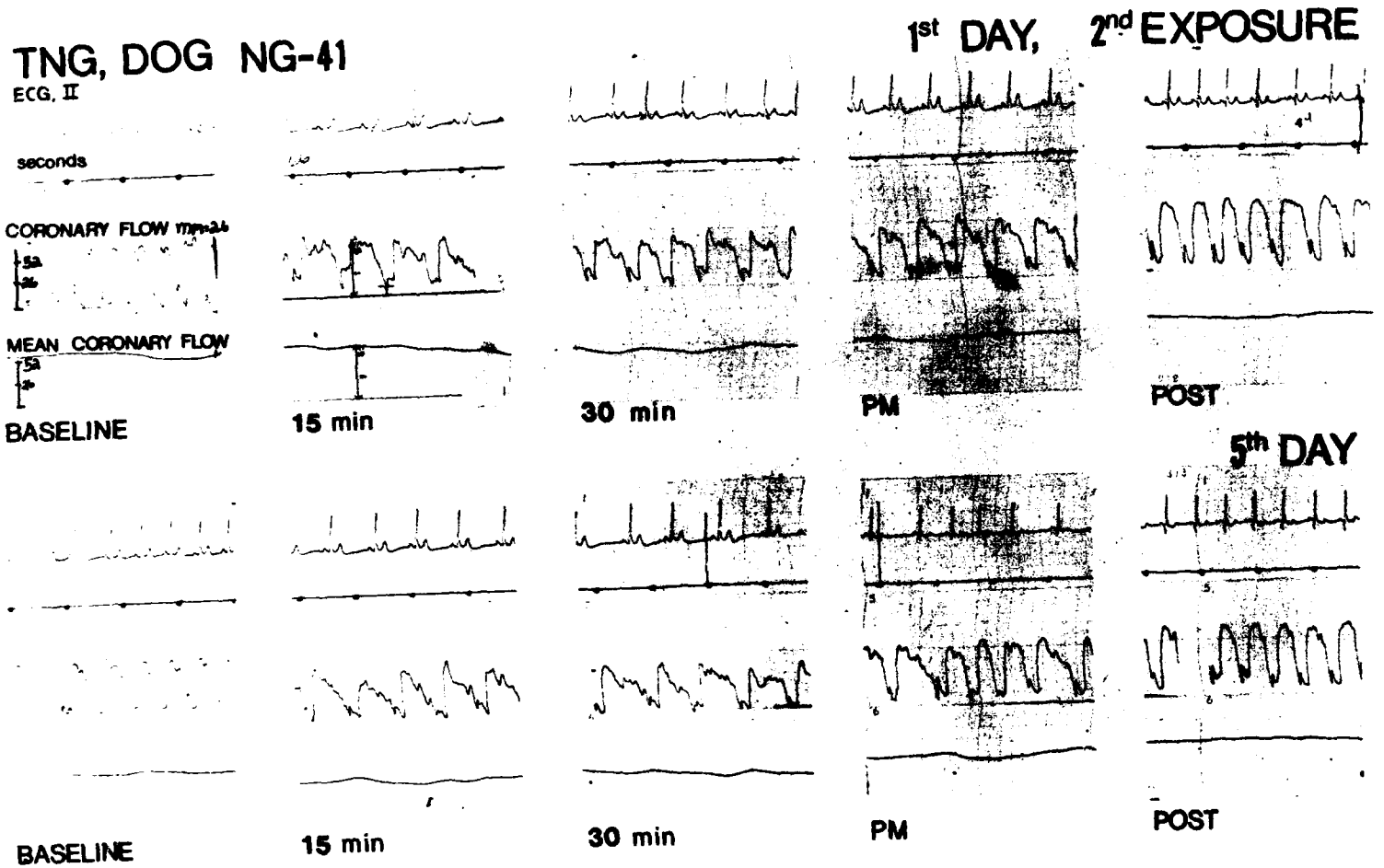


FIGURE 12(c) FIRST AND FIFTH DAYS, SECOND EXPOSURE SERIES

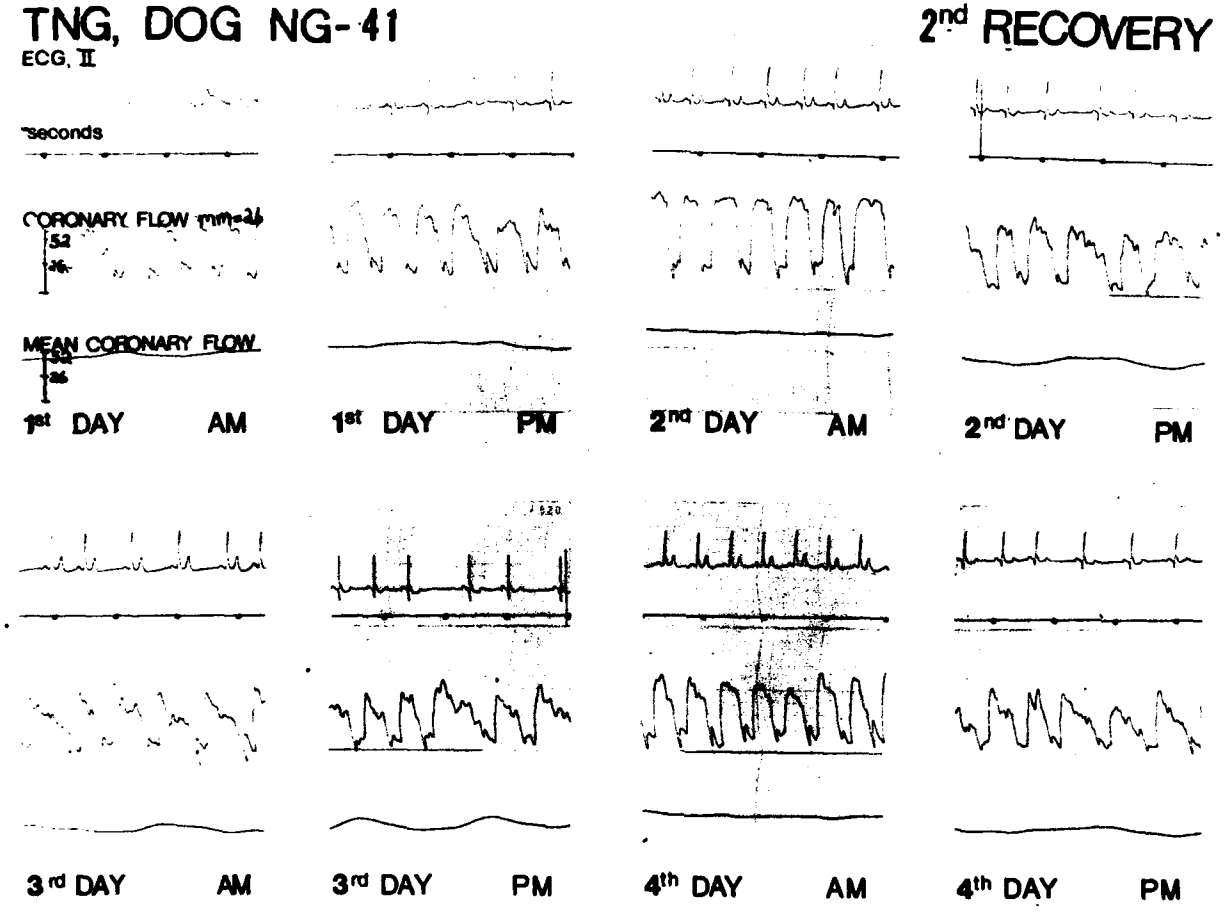


FIGURE 12(d) RECOVERY DAYS, SECOND SERIES

Dog NG-43 was implanted with telemetry devices, but only the pressure package functioned normally, so just LVP, heart rate, and ECG were recorded.

Initially, control data were collected for a 13-day period (interrupted by the Labor Day weekend); Table 17 summarizes these data. The collection periods each day corresponded to the approximate time the data would be collected if the dog were being treated. In general, the LVP remained between 120 and 124 mm Hg. Heart rates were generally 78 and above. Therefore, no patterns of pressure or heart rate changes were apparent that were consistent with the time of day. Figure 13 shows some of the representative control data collected from NG-43.

Table 18 presents the data collected from dog NG-43 during two TNG exposure-recovery sequences. Generally, during the first exposure series there was no change in LVP except for a possible slight decrease during 4 of the last 5 days. Heart rate dropped immediately after application of TNG each day except on Days 7 and 9. During the second exposure series, the LVP showed a slight drop on Day 5 as compared with Days 1-4, but on Days 6-9 there was a considerable overall pressure increase. The heart rate slowed after application of TNG on Days 1, 2, 3, and 5 only. Unfortunately, the power supply failed before the second recovery period.

Figure 14 shows the representative data collected from dog NG-43 during treatment and recovery. Most noticeable is the shift in the ECG to a pattern of T-wave inversion by the eighth treatment day. On the first day of the second exposure series there was even an occasional extrasystole and runs of ventricular tachycardia. This ECG pattern appeared frequently enough in the other dogs (5 of the last 6) to suggest that it is treatment-related.

Table 17

LEFT VENTRICULAR PRESSURE (LVP) AND HEART RATE
IN DOG NG-43 OVER A 13-DAY CONTROL PERIOD

Day	LVP (mm Hg)	Heart Rate (bpm)
1	60	102
	112	90
	116	102
	120	72
2	124	96
	120	78
	112	84
	120	84
	120	72
3	120	84
	124	84
	124	96
4	124	96
	124	96
	124	90
	124	96
	124	90
5	120	96
	128	84
	132	90
	132	102
	124	84
6	112	90
	124	90
	124	90
	124	102
	120	90
7	120	96
	120	78
	120	72
	120	84

(Continued)

Table 17 (Concluded)

Day	LVP (mm Hg)	Heart Rate (bpm)
8	124	120
	124	109
	124	72
12	120	78
	120	60
	124	66
	124	78
13	124	84
	124	84
	124	78
	124	78

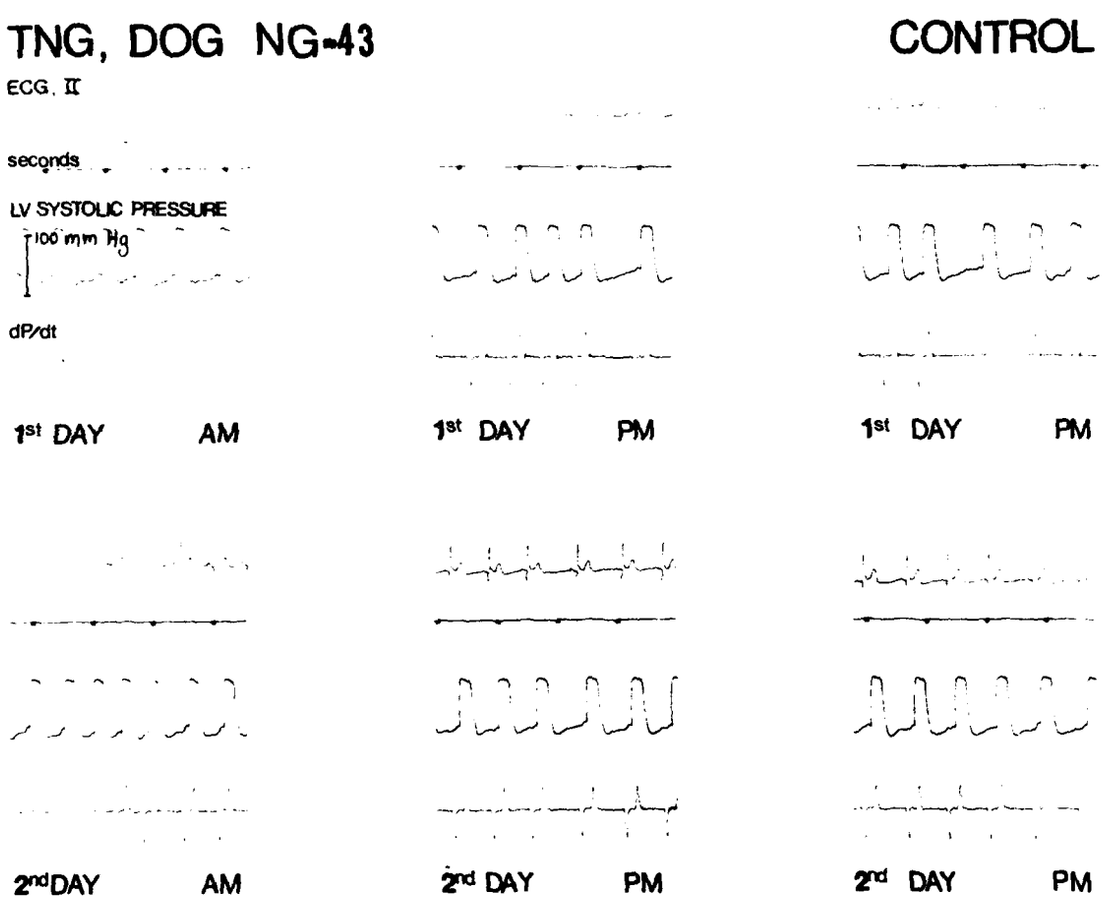


FIGURE 13 *CONTROL DATA COLLECTED FROM DOG NG-43 OVER SEVERAL DAYS PRIOR TO THE BEGINNING OF TREATMENT WITH TNG*

(a) Days 1 and 2

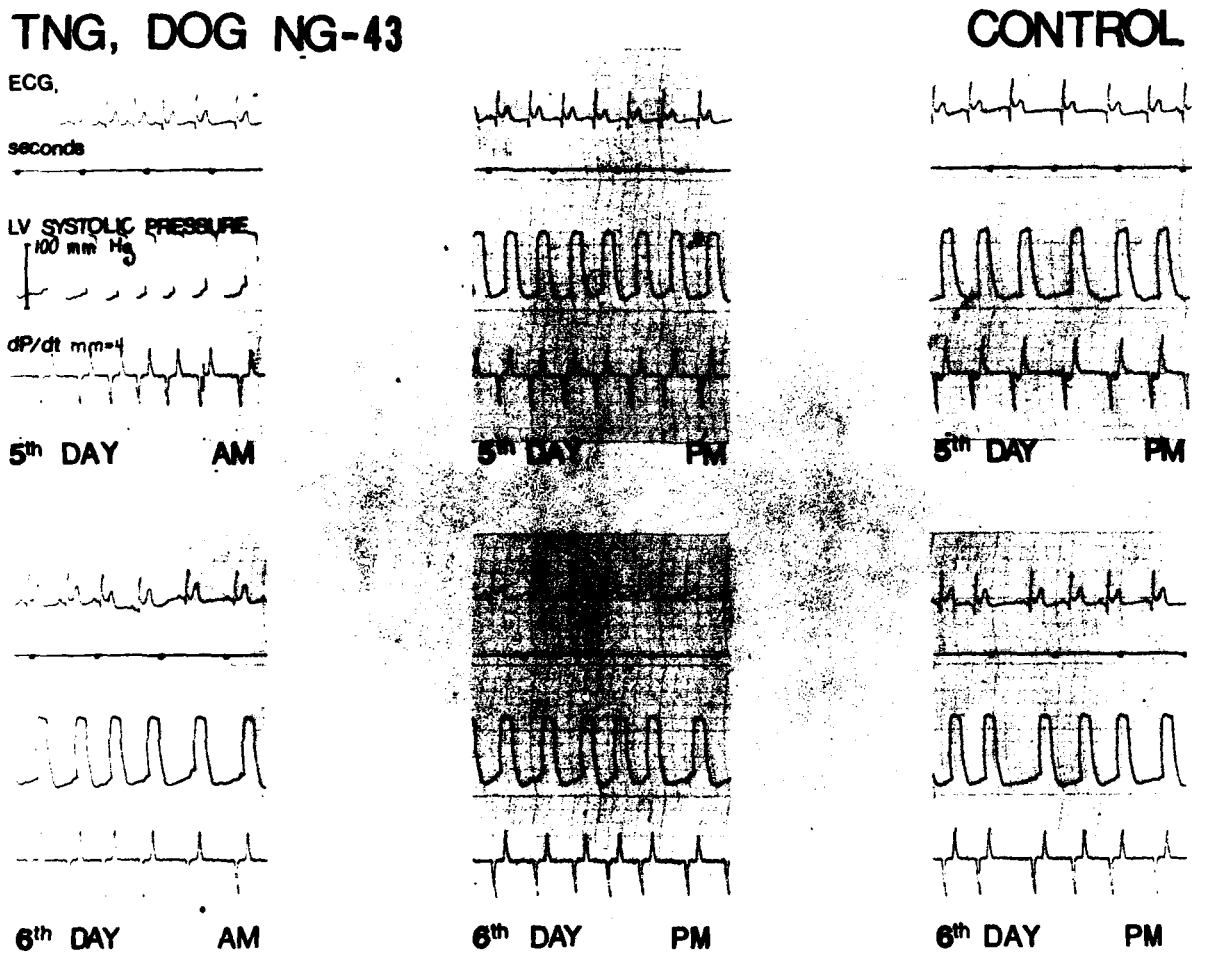


FIGURE 13(b) DAYS 5 AND 6

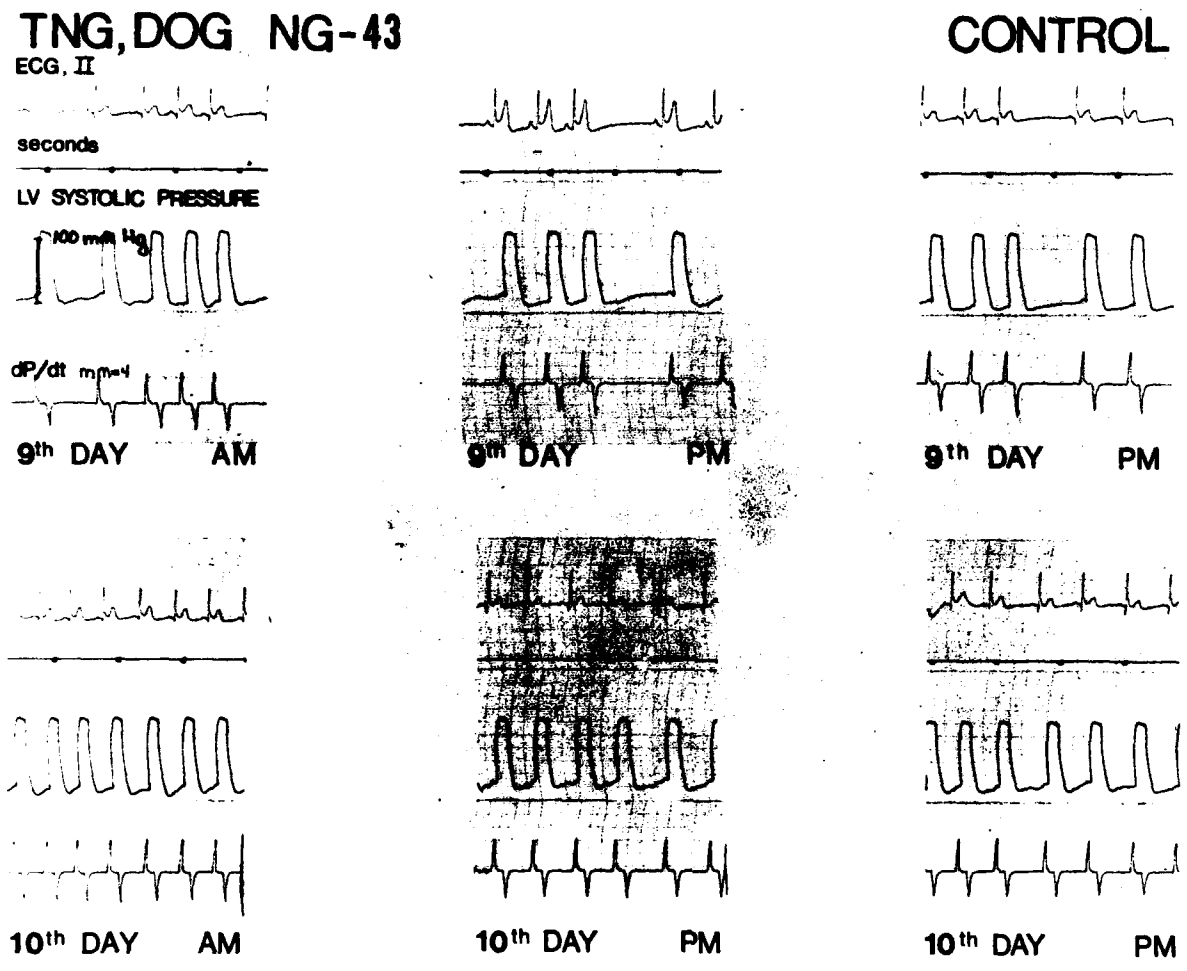


FIGURE 13(c) DAYS 9 AND 10

Table 18

LEFT VENTRICULAR PRESSURE AND HEART RATE IN DOG NG-43 DURING 10 DAILY EXPOSURES TO 10 g OF TNG ON LACTOSE AND DURING 4 DAYS OF RECOVERY*

Treatment Day	1st Exposure		2nd Exposure	
	LVP	HR	LVP	HR
1	124	84	120	114
	120	--	116	102
	120	72	120	108
	124	84	112	60
	124	76	116	138
	--	--	116	144
2	124	96	112	114
	124	84	116	108
	120	78	116	108
	124	60	120	60
	120	78	124	72
	120	96	124	96
3	124	96	120	132
	116	90	104	102
	124	78	116	84
	124	84	120	72
	124	84	120	96
	--	--	116	90
4	128	120	108	96
	124	66	116	96
	128	60	116	90
	128	96	116	90
	124	84	116	96
	124	66	116	90
5	120	96	104	90
	124	78	116	78
	124	60	116	72
	124	78	116	84
	120	84	112	84
	124	72	112	78

Table 18 (Continued)

Treatment Day	1st Exposure LVP	1st Exposure HR	2nd Exposure LVP	2nd Exposure HR
6	120	90	96	78
	120	72	132	84
	124	96	136	90
	120	66	128	90
	116	72	136	90
	120	78	136	72
7	120	84	132	102
	120	96	140	102
	120	102	140	96
	116	72	136	108
	116	78	144	102
	120	72	144	126
8	124	84	144	--
	124	78	144	--
	124	90	140	102
	124	72	132	132
	116	78	144	126
	116	78	--	--
9	120	84	148	96
	116	90	144	108
	120	84	144	84
	112	60	144	72
	116	66	144	108
	112	108	152	--
10	112	126	EF+	
	120	114		
	124	78		
	116	72		
	120	90		
	120	78		

(Continued)

Table 18 (Concluded)

Recovery Day	1st Exposure LVP	1st Exposure HR	2nd Exposure LVP	2nd Exposure HR
1	120	114	EF	120
	104	96		96
	120	66		90
	116	84		
	120	96		
	124	108		
	120	102		
	120	96		
2	120	108	EF	96
	116	126		
	116	102		
	120	96		
	116	126		
	116	114		
	112	84		
3	116	114	EF	90
	116	90		84
	116	90		90
	116	114		
	116	90		
	116	102		
4	114	102	EF	120
	116	66		102
	116	120		96
	116	96		
	116	96		
	116	84		

* The daily readings, in order, are: baseline, 3-5 minutes after starting treatment, 15 minutes after, 0.5 hour after, 4-6 hours after, and 0.5 hour after the treatment ended.

† EF = electronic failure (battery).

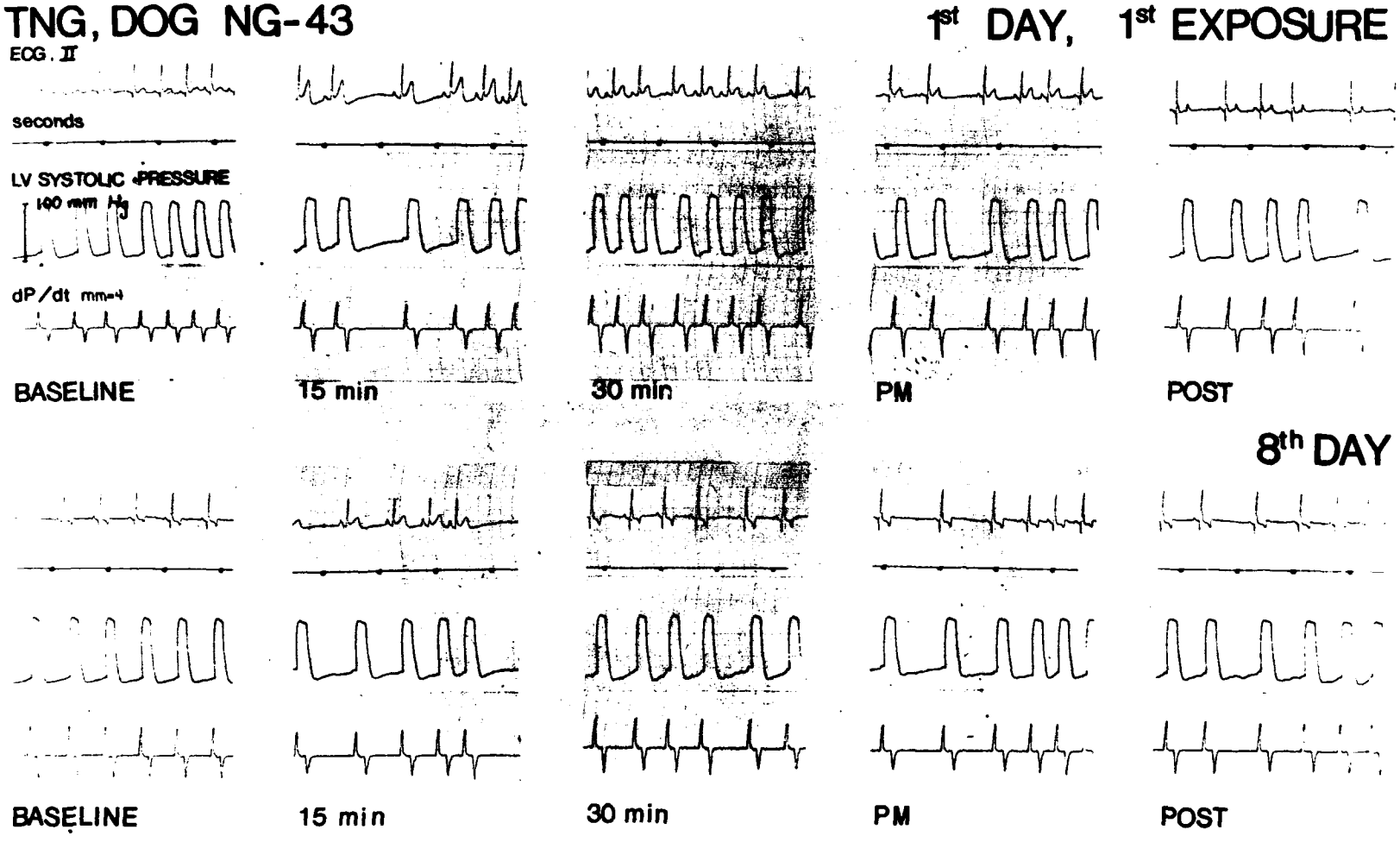


FIGURE 14 REPRESENTATIVE DATA COLLECTED FROM DOG NG-43 DURING TREATMENT WITH TNG AND DURING RECOVERY

(a) First and eighth days, first exposure series.

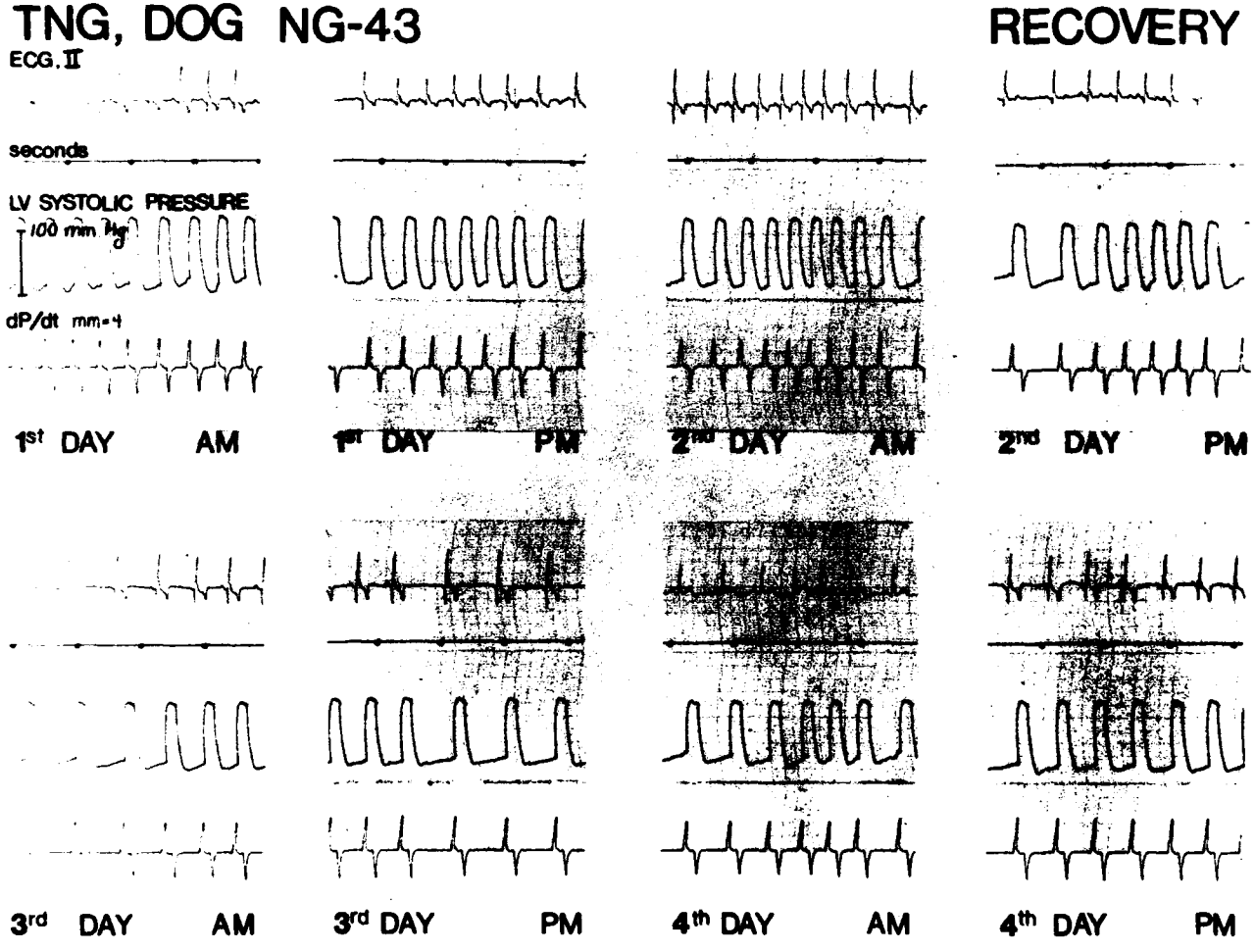


FIGURE 14(b) RECOVERY DAYS, FIRST SERIES

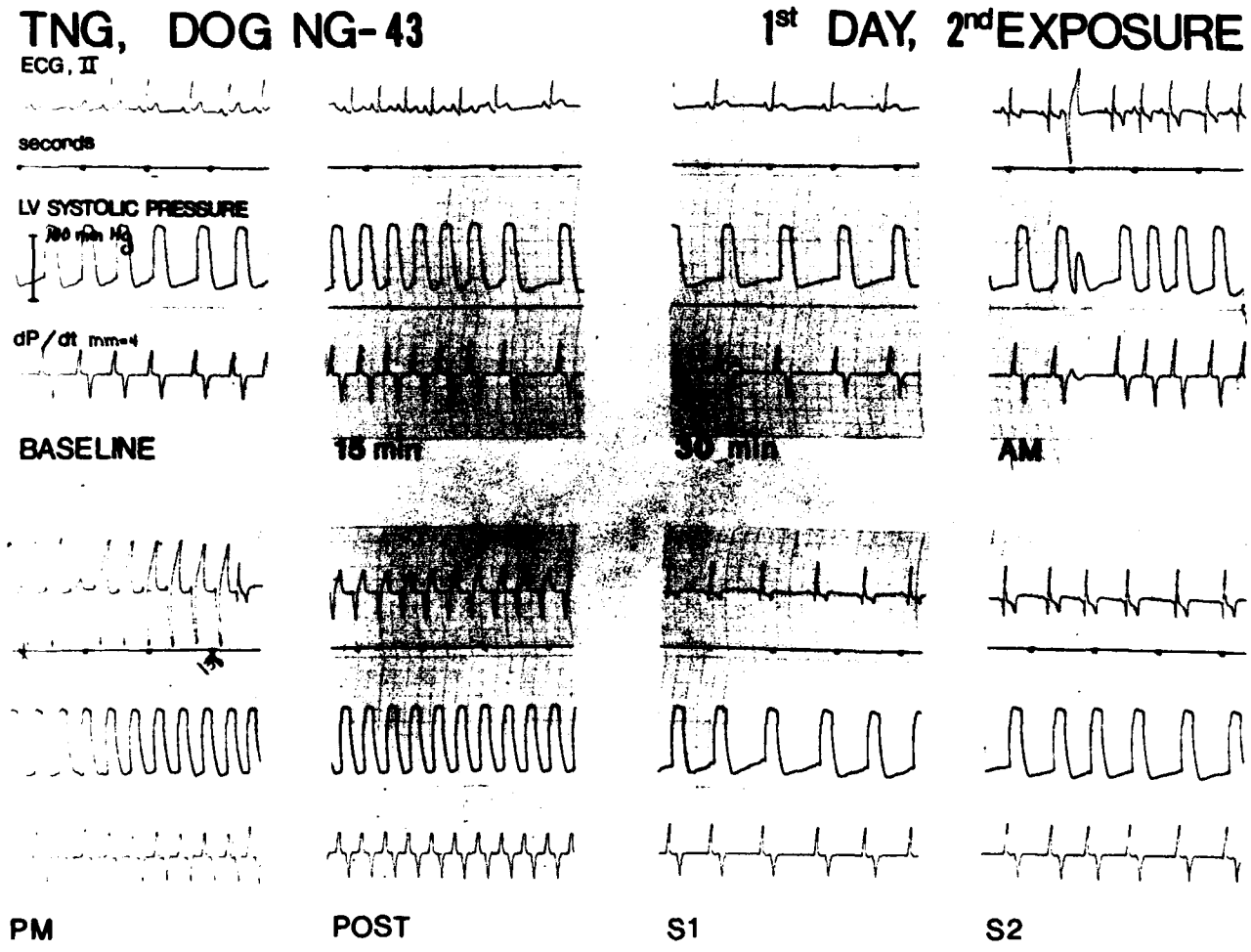


FIGURE 14(c) FIRST DAY, SECOND EXPOSURE SERIES

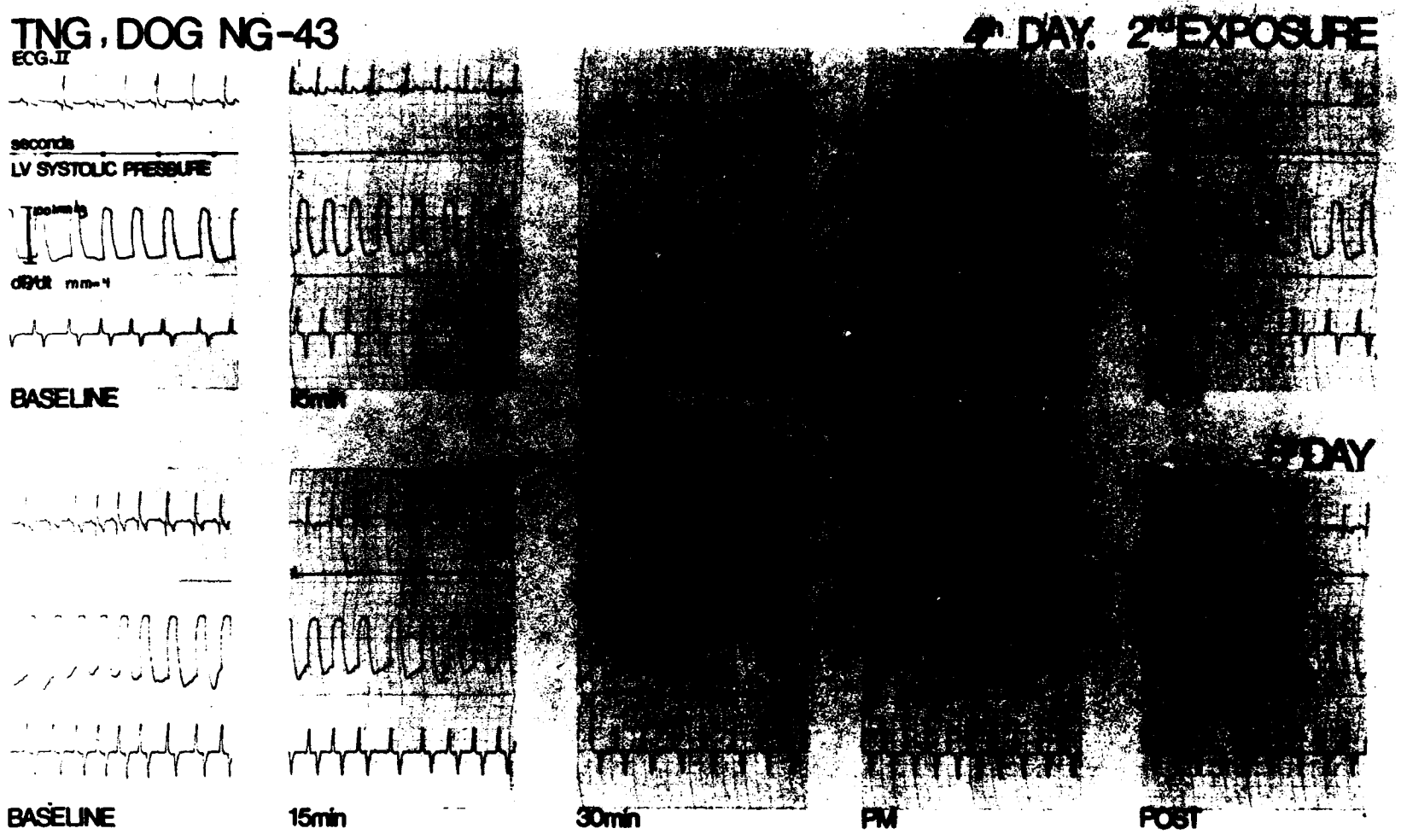


FIGURE 14(d) FOURTH AND EIGHTH DAYS, SECOND EXPOSURE SERIES

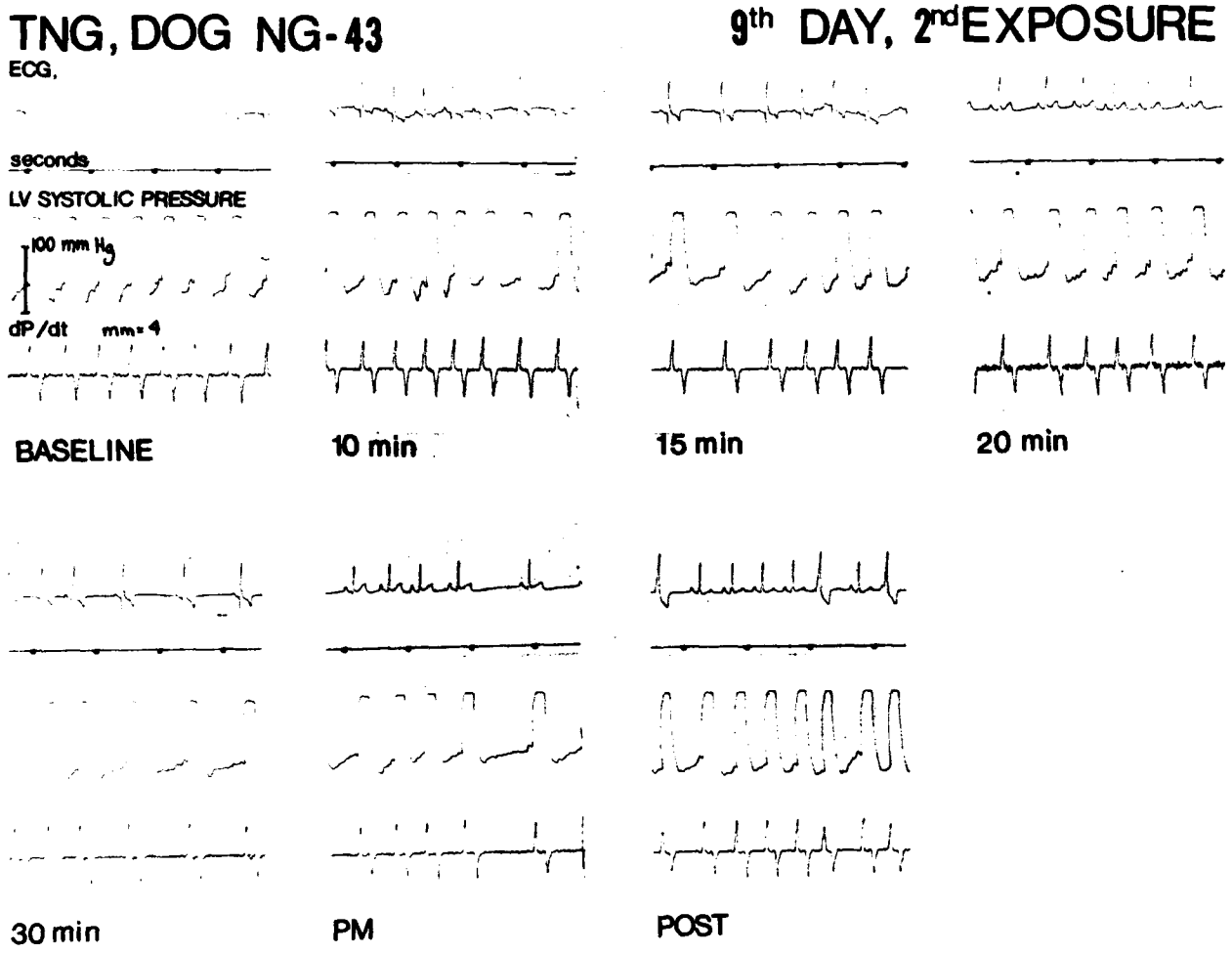


FIGURE 14(e) NINTH DAY, SECOND EXPOSURE SERIES

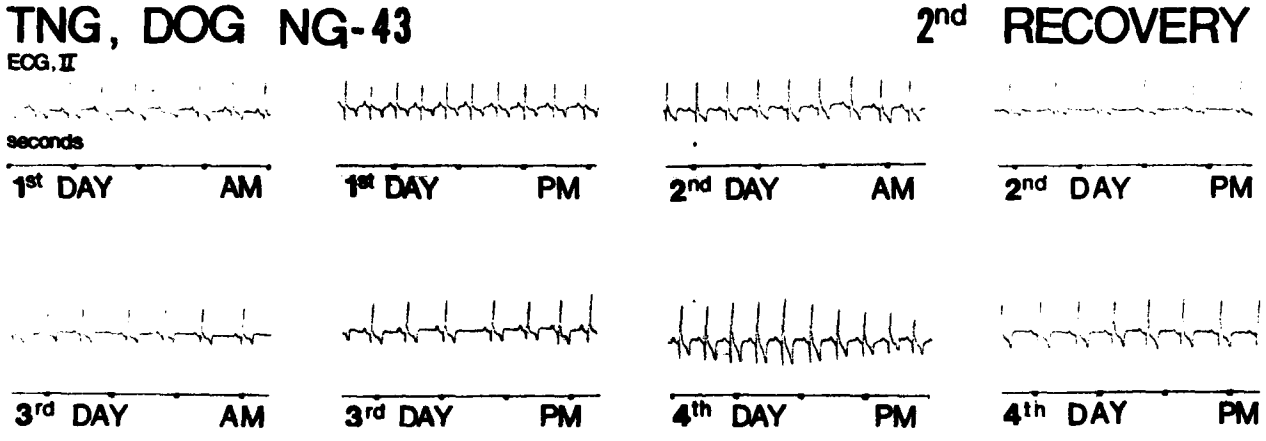


FIGURE 14(f) RECOVERY DAYS, SECOND SERIES

Biological Disposition Studies

Disposition of nitroglycerin in dogs was studied using tritiated nitroglycerin synthesized in this laboratory. The tritium label was on the #2 carbon atom.

Plasma levels of tritiated nitroglycerin were examined in dogs to determine whether nitroglycerin is handled differently after 10 daily exposures to its vapors. In an initial experiment, we determined the optimal time to sample for blood levels of the various nitroglycerins. The results of this study, shown in Table 19, indicated that most of the tri- and dinitroglycerins were degraded in less than 1 hour. Therefore, in the following studies blood samples were taken sooner and more frequently than in the initial experiment.

Dog NG-8 was injected with tritiated nitroglycerin (0.5 mCi) before and after 10 days of inhalation of nitroglycerin vapors. The results, presented in Table 20, indicate that the biological half-time ($T_{\frac{1}{2}}$) was somewhat longer after the 10-day treatment than before treatment. This is interesting because the half-time would be expected to be decreased, if anything, by enzyme induction or a similar mechanism. Because the post-inhalation assay was done 24 hours after the last inhalation exposure, no resident nitroglycerin would have been left to hinder the metabolic disposition of the tritiated nitroglycerin. Table 21 shows the data from four other dogs before they had been treated either by inhalation (NG-17) or by percutaneous administration of nitroglycerin. The data show that trinitroglycerin is rapidly converted to the dinitroglycerins *in vivo*. The half-time of 1,3-dinitroglycerin ranges from 24 to 45 min, and the half-time of 1,2-dinitroglycerin ranges from 22 to 34 min. The mononitroglycerins are more stable.

Radioactivity levels were determined in urine samples collected from four dogs during the 24 hours following injection. These data, shown in Table 22, indicate that 21 to 41% of the dose is excreted in the urine after intravenous injection of ^{3}H-TNG.

Table 19

DISTRIBUTION OF RADIOACTIVITY IN BLOOD FROM DOGS
AFTER INJECTION WITH 0.5 mCi OF TRITIATED NITROGLYCERIN

	Percentage of Radioactivity at Time (hours):			
	1	2	3	4
TNG	11.3	6.5	3.9	6.1
1,3-DNT	5.1	3.9	7.7	2.3
1,2-DNG	23.7	11.6	21.0	4.4
MNG	43.6	66.9	53.9	72.4
Origin	16.2	11.1	13.5	14.8

Table 20

DISTRIBUTION OF COUNTS FROM INJECTED TRITIATED NITROGLYCERIN
BEFORE AND AFTER 10 DAYS OF INHALATION
OF NITROGLYCERIN VAPORS IN DOG NG-8

	Time (min) After ^{3}H-TNG Injection					
	10	20	30	60	120	$T_{\frac{1}{2}}$
Before 10-day exposure						
TNG	0.2	--	0.4	0.4	0.2	
1,3-DNG	1.4	--	1.0	0.6	0.3	49.8
1,2-DNG	12.2	--	6.0	1.4	0.6	25.3
MNG	11.3	--	16.2	17.8	11.1	
After 10-day exposure						
TNG	1.5	0.8	1.1	0.7	1.4	
1,3-DNG	3.6	4.9	4.1	1.8	1.5	66.3
1,2-DNG	29.8	22.2	17.4	9.2	3.3	35.3
MNG	8.3	15.8	18.1	22.9	17.9	

Table 21

DISTRIBUTION OF COUNTS FROM INJECTED TRITIATED NITROGLYCERIN IN DOGS NG-13, NG-15, NG-17, AND NG-19

	Time (min) After ^{3}H-TNG Injection					
	10	20	30	60	120	$T_{\frac{1}{2}}$
NG-13						
TNG	0.3	--	0.3	0.7	0.2	
1,3-DNG	2.5	--	1.1	0.6	0.2	31.7
1,2-DNG	8.6	--	5.2	1.8	0.3	22.4
MNG	10.2	--	11.5	11.7	8.1	
NG-15						
TNG	0.3	0.3	0.2	0.2	0.1	
1,3-DNG	5.7	4.8	2.8	2.1	0.5	32.3
1,2-DNG	7.2	7.9	7.4	2.4	0.7	29.7
MNG	15.0	13.2	14.3	15.8	10.9	
NG-17						
TNG	0.2	0.2	0.2	0.2	0.1	24.2
1,3-DNG	4.2	4.3	1.9	0.8	0.2	34.4
1,2-DNG	8.8	6.3	5.8	1.8	1.0	
MNG	14.3	12.4	14.3	12.4	9.3	
NG-19						
TNG	0.8	0.3	0.2	0.4	0.8	
1,3-DNG	4.9	1.7	3.2	1.1	0.7	45.2
1,2-DNG	10.2	5.6	3.8	2.1	0.6	29.0
MNG	13.2	15.6	16.6	15.0	9.7	

Table 22

PERCENT OF RADIOACTIVITY OF INJECTED ^{3}H-TNG RECOVERED IN 24-HOUR URINE SAMPLES

Dog No.	Percent of Injected Dose
NG-13	21
NG-15	41
NG-17	32
NG-19	36

Confidence Limits on Half-Times of 1,3-DNT and 1,2-DNG for Individual Dogs

The estimated half-times of 1,3-DNG and 1,2-DNG and confidence intervals on these half-times in several dogs, both before and after TNG exposure (by inhalation or percutaneous) are given in Tables 23 and 24.

These estimates and confidence intervals were derived by the following procedure:

- A linear regression of the natural log of concentration versus time was performed.
- Bounds on the slope of the regression line were obtained.
- The bounds on the slope of the regression line were translated into bounds on the half-time using the equation

  half-time = -ln 2/slope.

The significance of the observed lengthening of half-time after exposure to TNG was statistically evaluated using a two-sample t-test on the difference between the two slopes. The results are summarized in Table 25.

Table 23

CONFIDENCE INTERVALS ON HALF-TIME OF 1,3-DNG

Name of Dog	Before TNG Exposure			After Inhalation Exposure			After Dermal Exposure		
	90% Lower Bound	Point Estimate	90% Upper Bound	90% Lower Bound	Point Estimate	90% Upper Bound	90% Lower Bound	Point Estimate	90% Upper Bound
NG-13	24.27	31.88	46.70						
NG-19	25.23	44.84	201.03						
NG-21	30.77	80.33	---						
NG-8	43.97	44.79	45.62	38.27	67.43	384.19			
NG-17	26.07	31.29	39.12	103.67	***	---	29.91	67.30	---
NG-15	19.65	26.04	38.59	38.59			77.15	---	---

*** = Point estimate over 10,000.

--- = Infinite upper bound.

Table 24

CONFIDENCE INTERVALS ON HALF-TIME OF 1,2-DNG

Name of Dog	Before TNG Exposure			After Inhalation Exposure			After Dermal Exposure		
	90% Lower Bound	Point Estimate	90% Upper Bound	90% Lower Bound	Point Estimate	90% Upper Bound	90% Lower Bound	Point Estimate	90% Upper Bound
NG-13	20.21	22.21	24.65						
NG-19	23.04	28.84	38.58						
NG-21	32.97	53.68	144.45						
NG-8	15.71	25.64	69.73	31.52	35.10	39.59			
NG-17	23.67	29.78	40.17	29.72	38.98	56.60	11.49	34.35	---
NG-15	25.14	34.32	54.04				91.31	---	---

--- = Infinite point estimate upper bound.

Table 25

STATISTICAL SIGNIFICANCE OF LONGER
HALF-TIMES IN INDIVIDUAL DOGS

Dog No.	TNG Metabolite	After Inhalation Exposure t-Value	After Inhalation Exposure t-Prob	After Dermal Exposure t-Value	After Dermal Exposure t-Prob
NG-8	1,3-DNG	1.56	.12		
	1,2-DNG	1.23	.16		
NG-17	1,3-DNG	6.52	.005	2.05	.075
	1,2-DNG	1.58	.12	.18	.43
NG-15	1,3-DNG			4.76	.01
	1,2-DNG			4.05	.02

Considering the small number of observations on each dog, these results are impressive. The changes in the half-times of 1,3-DNG after inhalation exposure for NG-17 and of 1,2-DNG and 1,3-DNG after dermal exposure for NG-15 as compared with the pre-exposure values are statistically significant at the 5% level. The half-time of 1,3-DNG after dermal exposure for NG-17 is statistically significant at the 10% level. The half-times of 1,3-DNG after inhalation exposure for NG-8 and of 1,2-DNG after inhalation exposure for NG-17 are almost statistically significant at the 10% level.

Statistical Significance Across Dogs

The statistical results stated so far have been for individual dogs. It is also possible to state statistical results across dogs, using the t-prob values in Table 25 and a technique developed by R. A. Fisher (7). Since for the individual dogs each metabolite had longer half-times after TNG exposure, it was expected that the overall significance levels (Table 26) would be considerably smaller than the individual significance levels.

Table 26

OVERALL SIGNIFICANCE LEVELS

	Inhalation Exposure			Dermal Exposure			Inhalation or Dermal Exposure		
	Chi-square	d.f.	Prob	Chi-square	d.f.	Prob	Chi-square	d.f.	Prob
1,3-DNG	14.84	4	.005	14.39	4	.007	29.23	8	.002
							(18.99)	(6)	(.005)
1,2-DNG	7.91	4	.05	9.51	4	.05	17.42	8	.025
							(14.07)	(6)	(.03)
TNG	22.75	8	.005	23.90	8	.005	46.65	16	.002
	(9.48)	(4)	(.05)	(11.15)	(4)	(.025)	(16.03)	(6)	(.015)

Temporarily ignoring the numbers in parentheses, observe that all results are statistically significant at the 5% level.

The technique developed by R. A. Fisher requires certain independence assumptions, which are statisfied for the four entries in the upper left-hand corner of Table 26. For the remaining entries, these independence assumptions will be somewhat violated because the same dogs are being used for both metabolites and one dog is used for both dermal and inhalation exposures. The numbers in parentheses represent conservative bounds on the significance levels. It may therefore be stated that, conservatively, this experiment has demonstrated at the 1.5% significance level that the metabolite half-times are lengthened by TNG exposure.

Nitroglycerin Levels in Blood After Percutaneous Administration of ^{3}H-TNG

Dogs NG-33 (29.5 kg) and NG-37 (22.7 kg) were each treated with 50 mg of TNG that contained 25 mCi of ^{3}H-TNG. The dogs were prepared for the dermal application of ^{3}H-TNG as follows. An area 10 cm^2 between the shoulders was clipped free of hair, shaved, and bordered with vasoline. The area was then covered with aluminum foil, which was taped to the skin.

The 50-mg dose of TNG was injected through the aluminum foil, and the hole made by the needle was covered with a piece of tape. The patch was covered with roller gauze wrapped around the dog's chest. Blood samples (2 to 3 ml) were collected every half hour.

After 5 hours, the patch was removed and soaked in 250 ml of 95% ethanol to recover the unabsorbed ^{3}H-TNG. The skin area under the patch was rinsed with gauze wetted with alcohol, and the gauze was soaked in 250 ml of 95% ethanol. The two alcohol rinses were combined, and aliquots were counted to determine the total radioactivity recovered from the application area.

The dogs were placed in individual metabolism cages for collection and deposit of urine during the next several days. Blood samples were taken during the 5-hour percutaneous exposure and at intervals during the week after exposure.

Immediately after collection, each blood sample was mixed with 0.5 ml of 5% mercuric chloride to prevent the further degradation of TNG. A small aliquot (40 µl) of the sample was counted to obtain total radioactivity. To the remainder, a mixture containing 40 µg each of TNG, 1,2-DNT, 1,3-DNG, 1-MNG, and 2-MNG was added as carrier. The resulting mixture was extracted twice with 5 ml of diethyl ether; the extraction was taken to dryness under nitrogen. The residue was redissolved in 100 µl of ether, and an aliquot (20 µl) was spotted on a silica gel plate. The plate was developed twice, using benzene-ethyl acetate (4:1) as the solvent. The developed plate was autoradiographed at -80° C for 1 week after the plate was treated with a 2,5-diphenyloxazole solution to sensitize the X-ray film. The developed X-ray film revealed radioactive spots of MNG only. The plate was then sprayed with a diphenylamine solution (20 ml of alcoholic 10% diphenylamine, 100 ml of concentrated HCl, and 80 ml of acetic acid) to permit visualization of the nitroglycerin spots. Areas corresponding to TNG, 1,2-DNG, 1,3-DNG, and MNG (the two MNG isomers are not separated in this system) were scraped into scintillation vials. The material obtained was wetted with 0.1 ml of ethanol or water, and then 10 ml of Scintisol (Isolab Inc., Akron, Ohio) was added. The counting data were corrected for quench and counting efficiency to convert them to the nanogram equivalence of each compound. For the calculation of MNG, the incomplete extraction (60%) of this compound by ether was taken into account.

To obtain some indication of the degree of dermal absorption of TNG, the 24-hour urine samples and radioactivity remaining in the applied area were counted. As shown in Table 27, the uptake of radioactivity was greater in dog NG-33 than in NG-37. The blood data presented in Table 28 verify this view. The radioactivity in the

Table 27

RADIOACTIVITY UPTAKE DATA IN TWO DOGS
AFTER PERCUTANEOUS ADMINISTRATION OF ^{3}H-TNG

	Percent of Applied Dose	
	Dog NG-33	Dog NG-37
Radioactivity appearing in first 24-hour urine collection	7.1	6.1
Radioactivity remaining in the patch and on the applied area	1.2	4.2
Radioactivity in whole blood:		
5 hours	1.1	0.6
Day 3	---	0.3
Day 4	0.3	---
Day 6	---	0.1
Day 7	0.1	---

Radioactivity in the blood after a few days is nonextractable; presumably it is in the body water. Blood volume was 2065 ml for Dog NG-33 and 1589 ml for dog NG-37.

Table 28

BLOOD LEVELS OF NITROGLYCERINS IN DOGS
AFTER PERCUTANEOUS ADMINISTRATION OF ^{3}H-TNG

		Nanograms per Milliliter of Blood			
	Hours	TNG	1,3-DNG	1,2-DNG	MNG
Dog NG-33	0.5	0.3	1.1	3.6	3.6
(29.5 kg)	1	--*	5.5	12.8	20.8
	1.5	--	7.6	18.9	31.3
	2	0.3	8.1	8.1	46.3
	2.5	--	7.8	19.1	80.8
	3	--	11.5	23.6	50.4
	3.5	--	4.6	8.7	80.3
	4	--	2.9	3.8	93.9
	4.5	0.4	2.9	4.8	100.9
	5	0.2	5.2	10.8	101.8
Dog NG-37	0.5	--	--	0.4	--
(22.7 kg)	1	--	0.6	1.7	1.7
	1.5	--	2.1	4.0	5.2
	2	--	2.5	4.1	8.2
	2.5	--	4.3	6.2	10.1
	3	--	4.6	5.3	18.1
	3.5	--	7.9	10.6	24.9
	4	--	6.9	9.8	23
	4.5	--	9.3	11.6	34.1
	5	--	9.2	13.9	37.5

* -- Indicates that radioactivity in the spot was less than twice the background counts.

blood, which must be mostly tritiated water after the first day, appeared to decrease, with a half-time of 2 to 3 days.

Essentially no TNG was present in the blood during the experimental period (5 hours). The bulk of the nitroglycerin present was mononitroglycerin. The level of dinitroglycerin in the blood approached that of mononitroglycerin only during the first few hours.

Routine Analysis for TNG in Blood

Initial analysis for TNG in blood was done by gas chromatography (GC), using the same column and conditions that were successful in analyzing atmospheric samples. We collected the blood in mercuric chloride, extracted it with ether, which was then evaporated, and diluted the final residue with ethyl acetate.

Blood was collected from dogs during percutaneous treatment with either 1.0 or 2.0 g of 10% TNG on lactose. Samples were taken before treatment and after 2 or 6 hours of treatment. These results are shown in Table 29. At first, it appeared that we could detect rather low levels of TNG by this method. However, there were many interfering substances that were extracted from the blood besides the nitroglycerins, so these initial results were probably erroneous. (It is possible that these peaks initially identified as TNG were actually one of the DNG isomers.) An additional problem encountered was that the extracts contaminated the GC column and this limited its use to 2 to 3 samples per day, after which the column had to be extensively reconditioned.

Further attempts were made to analyze for TNG in the blood by drawing the blood directly into mercuric chloride (to immediately stop any further enzymatic degradation of TNG) and then using a porasil column in an effort to separate the many polar components from the TNG. We still had contamination problems and still could not detect any TNG in the blood from the percutaneous treatment.

Table 29

BLOOD LEVELS* OF TNG DURING PERCUTANEOUS TREATMENT WITH 10% TNG ON LACTOSE†

Sample Time (hours)	2.0 g	1.0 g
0	0	0
2	0.13-0.86	0.14-1.14
6	0.97-1.44	0.20-0.64

* In µg/ml.
† Actually 100 mg of TNG per gram of material.

Several attempts were made to identify TNG in blood using the liquid chromatography/thermal energy analyzer (LC/TEA) system. Blood was collected on different days from NG-43 after one hour of treatment with 10 g of 10% TNG on lactose. In addition, the blood was collected directly into a silver nitrate solution instead of mercuric chloride. Again, no TNG was detected, but 1,2-DNG was identified in a concentration of approximately 2 µg/ml of blood.

In a further attempt to identify TNG in blood using the LC/TEA system, a dog was given an oral dose of 350 mg/kg of 10% TNG on lactose and then blood samples were collected at various times. These results are shown in Table 30. TNG was found only at 10 min after dosing. However, the continuing build-up of the DNGs and the mono isomers over the 3 hours indicated that absorption was continuing. Therefore, it appears that the metabolism of TNG to its isomers is very rapid in the dog.

In a similar study being conducted on another project, a rhesus monkey was treated percutaneously with a 2% ointment of TNG at a dose of 20 mg/kg. Blood samples were collected at various times after the start of treatment and analyzed using the LC/TEA system. These results are shown in Table 31. There were detectable levels of TNG

Table 30

BLOOD LEVELS* OF TNG AND ITS ISOMERS IN A DOG
AFTER ORAL DOSING WITH 350 mg/kg OF 10% TNG ON LACTOSE

	Time After Dosing (min)										
	10	20	30	60	90	120	150	180	240	300	360
TNG	13	ND†	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DNG	98	129	113	230	354	948	667	1746	699	322	233
1,2-DNG	212	277	215	415	699	1877	1265	3293	1216	444	334
Mono isomers	ND	30	148	434	277	1813	1362	2414	2654	2217	2299

* Concentrations are given in µg/ml of blood.

† ND = None detected.

Table 31

BLOOD LEVELS* OF TNG AND ITS ISOMERS IN A MONKEY AFTER PERCUTANEOUS DOSING WITH 20 mg/kg TNG†

	Time After Dosing (min)					
	60	120	180	240	300	360
TNG	ND‡	ND	ND	154	63	ND
1,3-DNG	3.2	44	28	640	68	14
1,2-DNG	1.7	141	70	1024	103	20
Mono isomers	--	--	171	3622	411	187

* Concentrations are given in µg/ml of blood.
† As a 2% ointment.
‡ ND = None detected.

in the blood only after 4 hours of treatment and the level had decreased to about one third of that concentration in 5 hours. It appears, therefore, that the dog and the monkey both metabolize TNG very rapidly and produce about twice as much 1,2-DNG as 1,3-DNG. Recent reports in the literature (e.g., Ref. 8) also indicate that no TNG was found in rat serum after percutaneous administration of TNG. Perhaps we have been looking for something that was presumed to be there but actually was not.

Pathology

Only one dog presented evidence of rejection of the implanted coronary flow probes. The remainder of the implants were well tolerated and no histological evidence of encroachment on the inner diameter of the artery was seen. The artery diameters were measured after fixation in formalin and before embedding and staining.

Problems with the pressure transducers were limited to clots forming over the implanted transducer surface. However, this seemed to occur within a few hours after implant or not at all. At least

two postoperative deaths were attributed to emboli breaking off from the newly implanted probe.

Statistical Analysis

Statistical analysis was limited to the data generated from the tritiated nitroglycerin experiments. Although an elaborate analysis was planned for all the pressure and flow data, the results were somewhat limited, and further statistical treatment would not shed much light on the results.

DISCUSSION

Inhalation exposures to nitroglycerin are difficult to assess in terms of the total dose being given because of the wide variation between the concentrations going into the chamber and those found in samples taken from the chamber itself or from the chamber exhaust. All the analytical samples were analyzed by the same gas chromatographic technique, so the differences detected are not likely to be analytical errors. Probably the nitroglycerin was being absorbed on the dog's fur, although some adsorption to the chamber walls may also have occurred. In fact, this is true of many organic vapors that we have used in other inhalation studies.

Several methods were considered to increase the nitroglycerin concentration in the chambers, including warming the generators and adding more generators. However, our consulting explosive experts strongly advised us to avoid both these options because we could cause some precipitation of nitroglycerin and create an extreme safety hazard. Since our workplace environment permits dermal exposures as well as inhalation exposures, it seemed reasonable to shift to percutaneous administration in order to increase the dose. Therefore, the remainder of the studies were done by percutaneous exposures (except for the special dose-response and ^{3}H-TNG studies).

Nitroglycerin inhalation on a daily basis either caused an increase in coronary flow during the first part of the 10-day exposure regimen or had no effect during most of the treatment. (However, there was always a treatment-related increase in coronary flow, lasting for a minute or so, at the very beginning of each daily inhalation exposure.) Coronary flow tended to decrease slightly on the last 2 or 3 days of treatment and appeared to be much less after the end of the 10-day treatment period in those dogs in which we were able to obtain measurements.

Intravenous injections of nitroglycerin were made in an instrumented dog to determine the minimum dose necessary to elicit a positive response in coronary flow. This was found to be 0.125 mg in a 10-kg dog. This particular study also confirmed that our experimental model was valid for studying coronary artery flow during nitroglycerin administration.

The main thrust of the study was carried out using 10 g of 10% nitroglycerin on lactose administered percutaneously for 6 hours daily for 10 days. The dogs were then closely observed during a 4-day recovery period. This was followed by a second 10-day treatment and 4-day recovery period. These dogs were instrumented with completely implanted telemetry packs so that we could collect data on ECG, left ventricular pressure, and mean and pulsatile coronary flow. The rationale for these studies was that (a) they provided two "withdrawal" periods for each dog, (b) we could see the interaction of pressure and flow patterns, and (c) we could also monitor any changes in cardiac electrical activity. The flow and pressure changes that we saw were minimal in all these dogs, although there were some interesting daily observations at the beginning of treatment each day. Very frequently we saw a drop in heart rate within 15 minutes after treatment started. This was unexpected, since any vasodilation that occurred as a result of the TNG exposure should cause a reflex increase in heart rate to maintain the pressure. There was also a slight fall in LV pressure. In addition, as the daily treatment progressed, the dogs became increasingly depressed in their activity and spent more of their time sleeping. For example, NG-43 was standing on the treadmill one day, went to sleep while standing, and fell on the floor. The mechanism for this depression of activity is unknown.

The interpretation of the coronary flow data, however, is limited by the lack of appropriate pressure, resistance, and contractility measurements. Thus, we can only speculate as to the nature of the changes observed in experiments such as the one illustrated in Figure 8. A literature review turned up at least one paper that is useful in interpreting our results. Vatner et al. (5) conducted a study of the

effects of TNG on conscious dogs. Figure 1 in their paper provides tracings obtained of the effects of iv TNG on arterial pressure, coronary flow velocity, and heart rate. The tracing presented for coronary flow velocity by Vatner and his colleagues is remarkably similar to the tracing presented in panel B in Figure 8. An initial increase in flow is followed by a slight secondary rise. They determined that the initial peak was the result of TNG effects directly on the coronary vascular smooth muscle, since this effect preceded any other hemodynamic alteration. The initial increase in coronary flow was soon followed by a hypotensive action of TNG, which caused reflex increases in heart rate and ventricular contractility. The latter responses in turn caused the secondary increase in coronary flow.

The article by Vatner et al. (5) not only provides an abundance of information regarding the acute hemodynamic actions of TNG, but also serves to confirm the validity of our coronary flow model.

Throughout the entire study, the most consistent finding in all the dogs was a gradual shift in the ECG to an inverted T-wave, often followed by arrhythmias and premature ventricular contractions, occasionally occurring in a bigeminal pattern. Whatever the cause of these changes, they were not reversible upon withdrawal from the treatment during the period studied. One theory was that they might be due to the implants on the coronary artery or to the pressure transducer in the left ventricle. However, one dog (NG-43) was observed for two weeks prior to treatment and did not exhibit these signs until after TNG treatment had begun. Therefore, the ECG changes seem related to the treatment.

Exercising the dogs by trotting them on a treadmill did not produce any unexpected changes in coronary flow or ventricular pressure. However, the arrhythmias and the inverted T-wave disappeared for several minutes after stress, and this phenomena has been observed by others as well (13).

A very extensive effort was exerted to try to identify and quantitate TNG in the blood from dogs while they were being treated. We were convinced that it was there and spent many hours trying to identify it. Yet our gas chromatograph failed to detect it. Also, we did not find it using the liquid chromatography/thermal energy analyzer system, nor did we detect more than traces using ^{3}H-TNG. This is in agreement with a report by Yap and co-workers (10,11), who could not find TNG in plasma after 7 to 14 mg/kg was applied topically. Apparently the absorption is slow enough so that it is all metabolized as it enters the blood, or else it is metabolized by enzymes in the subcutaneous tissue. Yet, based on the large quantity of dinitroglycerins and mononitroglycerins identified in the blood and urine in both the LC/TEA and ^{3}H-TNG experiments, we know that absorption does occur.

An interesting finding was that the biological half-time of the nitroglycerins increased after 10 days of daily treatment. Although the significance of this observation is obscure, it has also been observed in men working in munitions plants (12). That report stated that the half-time of TNG is about 0.4 hr in unexposed men and 0.6 hr in munition workers.

CONCLUSIONS AND RECOMMENDATIONS

Conscious dogs instrumented with Doppler flow probes and pressure transducers are valid and useful experimental tools for studying the cardiovascular effects of repeated daily treatment with nitroglycerin. The use of telemetry implants permits the use of these animals in studies lasting weeks or months.

In this investigation attention was focused on TNG effects on coronary blood flow, left ventricular pressure, and cardiac electrical activity. In response to instructions from the Project Monitor, initial studies were carried out using beagles, which proved to be difficult to instrument because of their comparatively small size. The study was completed with specially bred mongrels. The switch in the choice of animal and the improvement in the reliability of the instrumentation provided to SRI on subcontract from Stanford University increased the amount of usable data obtained in the study.

TNG was administered via inhalation, percutaneous absorption, and in a few experiments by intravenous injection. The basic protocol in the chronic study was to expose the animal to TNG over a 6-hour period for 10 consecutive days followed by a 4-day recovery period. In some dogs exercise studies were included (mild treadmill trotting).

Based on the intravenous injection study, the minimum dose of TNG necessary to produce a cardiovascular response is approximately 12.5 μg/kg. Inhalation of TNG vapors produced a transient increase in coronary flow similar to that observed after intravenous injection. However, on continued inhalation no further evidence of vasodilation was evident, indicating that physiological adjustments had taken place to return coronary flow to normal levels. Also, there was an overall decrease in coronary flow after 10 days of treatment. Percutaneous daily application of up to 10 g of 10% TNG on lactose had minimal effects on coronary flow. Because aortic pressure measurements were

not obtained, we have no way of describing the effects of TNG on coronary resistance. Also, heart rate was not controlled in any experiment, making it impossible to determine whether the coronary flow effects, modest as they were, were due to a direct action of TNG or secondary to changes in ventricular myocardial requirements. It is doubtful that TNG administered via inhalation or percutaneous absorption would actually alter coronary flow, as was shown with intravenous administration. Delivery of the drug to the vascular smooth muscle receptors of the coronary system is just too slow to produce any observable acute effects.

The left ventricular effects of TNG are less difficult to interpret, although their validity is lessened by the absence of data on cardiac output, stroke volume, etc. In dog NG-34 (Figure 9) and Dog NG-43 (Figure 14) left ventricular end diastolic pressure (LVEDP) is clearly enhanced by TNG 15 minutes after the start of exposure in the first series of exposures. This response occurs when dilation of the capacitance vessels occurs, thus increasing venous return, and has been shown to occur with nitrate administration in several experimental studies. As expected, the response wears off, both during the same exposure and upon repeated exposure. This phenomenon is the clearest evidence of tolerance occurring in this study. Moreover, it does substantiate that enough TNG (or TNG metabolites) is present to exert a pharmacologic action.

We found no evidence of reflex vasoconstriction following withdrawal of several days of daily TNG treatment. The "sudden death" syndrome of munitions workers may be more closely association with some biochemical mechanism such as monamine oxidase inhibition and hypersensitivity to endogenous catecholamines. The most evident sign of subchronic toxicity in the study was the electrocardiographic changes observed. Sinus, atrial and ventricular arrhythmias were recorded. These are very likely drug related and not due to the instrumentation because of experience gained with similar instrumented

animals in other studies at SRI. No obvious electrocardiographic signs of myocardial ischemia were produced upon withdrawal of the drug. The T-wave changes (inversion, increase in voltage) occurring did not follow a pattern one would expect from ischemia and infarction. Because they were drug-induced and were not reversible following discontinuation of TNG, they should be studied in greater detail.

Based on the experiments conducted here, we have no basis for recommending any change in the present threshold limit value established by OSHA. We do recommend very close surveillance of munitions workers for any changes in their electrocardiograms. Also, additional studies of the electrocardiographic actions of TNG in chronic, unanesthetized preparations are indicated by our results.

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Appendix
PROBLEMS ENCOUNTERED

Several problems arose during the study. Because this was a developmental study, problems were expected but not specifically anticipated. The major problems were:

- Contamination of the microcircuitry laboratory during assembly of some electronic components delayed production of some of the implantable telemetry equipment.
- The biological life of the Doppler flow probes is about 60 days, instead of longer as had been thought. This caused loss of some animals before we could expose them to nitroglycerin.
- The dogs managed to chew the hard-wired probes with external leads, emphasizing the need for using the totally implantable packages.
- Magnetic switches on the implanted power supplies are not reliable and can be turned on and off by the dog jumping around or getting near magnetic fields undetected.
- This resulted in run-down batteries. Also, the batteries had a highly variable life-span. One lasted only 1 hr instead of the advertised 60 hr.
- Many minor electronics problems developed, most of which were solved, but with considerable time and effort.
- Whole-body inhalation exposure of dogs to vapors of nitroglycerin was accomplished. However, estimating the doses administered was extremely difficult because, under these conditions, the amount of nitroglycerin breathed by the animals cannot be regulated.

- Percutaneous absorption rates were difficult to estimate because we kept looking for blood levels of TNG when there was no TNG present.
- Many substances were extracted from blood that interfered with the gas chromatography analysis for TNG.
- A poorly operating respiratory pump caused some deaths of the dogs in surgery before we discovered its faulty operation.
- Blood clotting on the tip of the implanted pressure transducers occurred occasionally. The breaking loose of the clots probably caused an infarction on at least two occasions.
- The diameter of the coronary artery is difficult to determine. It increases when it is dissected away from the myocardium during surgery. It decreases when it is removed at necropsy and fixed in formalin.

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